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EFFECT OF ENTOMOPATHOGENIC FUNGI ON SUCKING PESTS AND LEAF FEEDERS OF VEGETABLES UNDER *IN VITRO* CONDITIONS

JINCY SIMON

Thesis submitted in partial fulfillment of the requirement for the degree of

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Faculty of Agriculture
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2002

Department of Agricultural Entomology
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM-695 522

DECLARATION

I hereby declare that this thesis entitled "**Effect of entomopathogenic fungi on sucking pests and leaf feeders of vegetables under *in vitro* conditions**" is a *bonafide* record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

Vellayani,
15-11-2002

JINCY SIMON

CERTIFICATE

Certified that this thesis entitled **"Effect of entomopathogenic fungi on sucking pests and leaf feeders of vegetables under *in vitro* conditions"** is a record of research work done independently by Ms. Jincy Simon under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

Vellayani,
15 -11-2002

Susamma Mathai

Dr. Susamma Mathai
(Chairman, Advisory Committee)
Professor
Department of Agricultural Entomology
College of Agriculture, Vellayani
Thiruvananthapuram.

ACKNOWLEGMENT

"Trust in the Lord with all your heart and lean not on your own understanding;
In all your ways acknowledge Him, and He will make your paths straight."

Holy Bible (Proverbs 3: 5 – 6)

I wish to express my heartfelt gratitude and indebtedness to Dr. Susamma Mathai, Professor, Department of Agricultural Entomology and Chairperson of my Advisory Committee for her valuable guidance, constant encouragement and everwilling help throughout the course of investigation. I pleasantly recall the moral support and affection shown to me by her during the entire period of study.

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APPROVED BY

CHAIRMAN

Dr. SUSAMMA MATHAI
Professor,
Department of Agricultural Entomology,
College of Agriculture, Vellayani,
Thiruvananthapuram-695 522

MEMBERS

Dr. K. SARADAMMA
Professor and Head,
Department of Agricultural Entomology,
College of Agriculture, Vellayani,
Thiruvananthapuram-695522.

Dr. T. JIJI
Assistant Professor (S.S),
Department of Agricultural Entomology,
College of Agriculture, Vellayani,
Thiruvananthapuram-695522

Dr. C. K. PEETHAMBARAN
Professor and Head,
Department of Plant Pathology,
College of Agriculture, Vellayani,
Thiruvananthapuram-695522.

EXTERNAL EXAMINER

Dr. M. S. PALANISWAMI
Principal Scientist and Head,
Division of Crop Protection,
CTCRI, Sreekaryam,
Thiruvananthapuram.

TO MY PAPPA

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LIST OF ABBREVIATIONS

kg	kilogram
g	gram
mg	milligram
ml	millilitre
m	metre
cm	centimetre
mm	millimetre
%	percentage
^{0}C	degree Celsius
Fig.	Figure
MAS	Months after storage
MCD	Mean colony diameter
PIOC	Percentage inhibition over control
PDA	Potato dextrose agar
CD	Critical difference
ppm	Parts per million
EC	Emusifiable Concentrate
h	hour
viz.	namely
et al.	And others
spp.	Different species

INTRODUCTION

1. INTRODUCTION

Use of chemical pesticides has become an indispensable component in modern day intensive agriculture, where pests and diseases pose a major threat to farmers. Several environmental and ecological hazards have already been reported as an outcome of widespread use of chemical pesticides in agriculture.

Occurrence of pests and diseases is a major problem in vegetable cultivation. Vegetables are consumed in more or less unprocessed form and therefore the problem of pesticide residue is more prominent in this case.

Incorporation of biological control methods, which impart minimum harmful impact on the environment and non-target organisms, is gaining importance nowadays. Practically, almost all insects have their natural enemies in the form of predators, parasitoids or pathogens. The identification and development of suitable natural enemies and their incorporation in integrated pest management programme against each pest is an important step in successful biocontrol method.

Under natural conditions, fungi often cause epizootics among the arthropod pests. Charnley (1989) recorded more than 700 species of entomopathogenic fungi. Fungi, being able to invade directly through the cuticle, offer an advantage over the bacterial and viral pathogens *ie.*, fungi can infect even the piercing and sucking insects.

Most of the fungal pathogens require high humidity and optimum temperature for their natural existence and virulence. The high humid condition in Kerala, offer a suitable environment for successful utilization of entomopathogenic fungi for pest management.

Though the entomopathogenic fungi belonging to Hyphomycetes and Deuteromycetes attack a wide range of insects falling in different orders, they exhibit considerable species specificity also. For example,

the white muscardine fungus *Beauveria bassiana* has more than 1000 isolates, each being specific to a particular host.

Studies on pathogenicity and virulence, mass production and formulation techniques, compatibility with other pest control methods especially the chemicals and stability of the fungus on storage are important in the development of a mycopesticide.

Considering all these parameters, the present study was undertaken to identify the host range of the entomopathogenic fungi *Beauveria bassiana, Beauveria brongniartii, Paecilomyces fumosoroseus, Paecilomyces lilacinus, Metarhizium anisopliae, Fusarium pallidoroseum* and *Rhizopus oryzae* among the major vegetable pests.

Identification of cheaper and locally available mass multiplication media, compatibility with commonly used pesticides in vegetables, dosage for 50 per cent mortality under laboratory conditions and effect on storage for a period of six months at room temperature were also studied.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Incidence of pest attack is a major constraint in vegetable cultivation. Vegetable crops are attacked by several lepidopteran, hemipteran and coleopteran pests. The use of microbial agents in the management of vegetable pests is gaining momentum in many countries. Under natural conditions, fungi are a frequent and natural mortality factor in insect populations. Unlike other potential biocontrol agents, fungi do not have to be ingested to infect their host but invade directly through the cuticle and so can be used for the control of all insects including the sucking pests (Ignoffo, 1978). Nearly 700 species of fungi under 90 genera are known to be entomopathogens (Charnely, 1989).

Entomopathogenic fungi are reported from most of the insect taxa like Lepidoptera, Isoptera, Coleoptera, Diptera, Hemiptera and Orthoptera. These fungi have a wide host range including many important pests of vegetable crops. The most widely studied entomopathogenic fungi belong to the fourteen genera namely *Beauveria*, *Metarhizium*, *Verticillium*, *Hirsutella*, *Erymia* (*Zoopththora*), *Nomuraea*, *Aspergillus*, *Aschersonia*, *Paecilomyces*, *Tolypocladium*, *Leptolegnia*, *Culicinomyces*, *Coelomomyces* and *Lagenidium* (Moore and Prior, 1993).

2.1 FUNGAL DISEASES OF VEGETABLE PESTS

2.1.1. *Beauveria* spp.

Beauveria spp. were used as entomopathogenic fungi as early in 1836 (Bassi) for the management of chinchbugs. However the earliest report of the use of this fungus in the management of vegetable pests was with the work of Klochko (1969) who found *Beauveria tenella* and *Beauveria bassiana* as efficient pathogens of *Epilachna vigintioctopunctata*. He found that the spores of *B. tenella* from naturally infected insects were more virulent than those from pure culture. The fungus caused 98 per cent mortality of larvae in 13 days.

B. bassiana caused 100 per cent mortality of sweet potato weevil, *Cylas formicarius*, *Chalcodermus ebeninus*, *Metamasius sericeus* and *Cosmopolites sordidus* (Diaz *et al.*, 1986) and reduced the field population of grasshopper *Melanoplus sanguinipes* by 60 per cent in nine days (Johnson and Goettel, 1993).

A homopteran isolate of the fungus when sprayed at 10^8 spores ml^{-1} caused 72 – 86 per cent mortality of green peach aphid *Myzus persicae* attacking canola (Miranpux and Khachatourians, 1993) and another isolate was found pathogenic to white grub *Holotrichia serrata* (Nehru and Jayarathnam, 1993).

Poprawski and Khachatourians (1994) reported white muscardine fungus as an important biocontrol agent of *Nilaparvata lugens, Spodoptera litura, Heliothis armigera, Holotrichia consanguinea* and *Schistocerca gregaria.* Extensive mortality of ovipositing females of grasshopper *M. sanguinipes* was reported by exposing them to sand containing *B. bassiana* at 7.2 x 10^6 to 4.2 x 10^7 conidia g^{-1} (Inglis *et al.*, 1995). They observed heavy infection of the fungus in the abdominal region of the insects.

Saxena and Ahmad (1997) recorded a negative correlation between the concentration of the fungal spores in spray fluid and the incidence of *Helicoverpa armigera* in chickpea. *B. bassiana* was found pathogenic to brinjal spotted beetle, *Henosepilachna vigintioctopunctata* (Padmaja and Kaur, 1998), *Aphis craccivora* (Zaki, 1998), white flies, *Trialeurodes vaporariorum* and *Bemisia argentifolii* attacking tomato (Olson and Oetting, 1999), eggs and nymphs of *B. argentifolii* (Orozco *et al.*, 2000 and Ramos *et al.*, 2000).

B. bassiana isolate MG-Gb-1 caused more than 95 per cent mortality of *Plutella xylostella* at 1 x 10^7 conidia ml^{-1} (Masuda, 2000) and another isolate P89 caused 100 per cent mortality of cabbage maggot, *Delia radicum* (Meadow *et al.*, 2000). The fungus prevented the egg laying of *D. radicum* when the other females were treated with it.

Ekesi (2000) reported higher per cent mortality and antifeedant effect of *B. bassiana* isolate CPD_3 and CPD_{10} on cowpea leaf beetle, *Ootheca mutabilis*.

2.1.2 *Fusarium* spp.

The entomopathogenic property of *Fusarium* was first reported by Kunckel d' Hercularis and Langlois (1891) on *S. gregaria*. The use of this fungus has not been commercially exploited as in the case of *B. bassiana*. *Fusarium equiseti* was observed to infect pupal and adult stages of okra petiole maggot *Melanoagromyza hibisci* and caused 100 per cent mortality of brinjal mealy bug *Coccidohystrix insolita* (Gopinathan *et al*., 1982).

Jacob *et al*. (1978) described the occurrence of *Fusarium moniliformae* var. *subglutinans* on the grubs, pupae and adults of epilachna beetle, *H. vigintioctopunctata*. The fungus at 7.5 x 10^5 conidia ml^{-1} caused 96.67 per cent mortality of *H.vigintioctopunctata* (Beevi, 1979). Under laboratory conditions, the same fungus was pathogenic to *Mylabris pustulata* and *Aulacophora* sp. (Beevi, 1982).

Entomopathogenic ability of other species of *Fusarium* have also been reported – *Fusarium semitectum* on *M. persicae* (Nagalingam and Jayaraj, 1986), *Fusarium pallidoroseum* on cowpea aphid *A. craccivora* (Hareendranath *et al*., 1987) and *M. persicae* (Rejirani, 2001).

2.1.3 *Rhizopus* spp.

Narayanasamy (1998) reported *Rhizopus oryzae* as a pathogen of the rice pest *N. lugens*. *R. oryzae* isolated from red cotton bug *Dysdercus cingulatus* was found to be pathogenic to the nymphs and adults of that pest at 280 spores ml^{-1} concentration (Hebsybai *et al*., 1999).

Mathai (1999) reported that *R. oryzae* at 5 x 10^6 spores ml^{-1} was pathogenic to *Leptocoriza acuta*, *Coptosoma cribaria*, *Phymatostetha deschampes*, *Nezara viridula*, *Paradasynus rostratus*, *Riptortus*

pedestris, *Anchon pilosum*, *Toxoptera aurantii*, *A. craccivora and Pseudococcus lilacinus*.

2.1.4 *Metarhizium* spp.

Metarhizium is a fungus which is being used as a commercial product for the management of many insect pests. The first report of this fungus as a entomopathogen can be traced to the work of Metchnikoff (1879) on wheat cockchaffer. However, reports of the use of fungus to manage vegetable pests are very scanty.

Chaudhuri (1976) reported the death of brinjal mealy bug *Centrococcus insolitus* in seven to ten days when inoculated with *Metarhizium anisopliae*. Gopalakrishnan and Narayanan (1988) reported 100 per cent mortality of mature larvae of noctuid *H. armigera* by *M. anisopliae* var. *minor*.

Fourth instar larvae of Colorado potato beetle, *Leptinotarsa decemlineata* were killed by the same fungus (Chabchoul and Taborsky, 1991). The fungus when applied on the larvae of sweet potato weevil, *C. formicarius* caused death of the larvae in three to five days after inoculation (Rana and Villacarlos, 1991). They recorded the adverse effect of fungal application on total fecundity also.

Finnish isolate of *M. anisopliae* caused 40 – 50 per cent mortality of second and third instar larvae of cabbage root fly, *Delia floralis* under laboratory and glasshouse conditions at 1.5 x 10^{10} spores ml^{-1} (Vanninen *et al*., 1999). Wickramatileke *et al*. (2000) evaluated the effectiveness of *M. anisopliae* var. *major* on cabbage semilooper, *Chrysodeixis eriosoma* and observed 90 per cent mortality within seven days after treatment.

2.1.5 *Paecilomyces* spp.

Paecilomyces farinosus, an entomogenous fungus was isolated from *L. decemlineata* by Bajan and Kmitowa (1969). When the field

collected eggs were sprayed with suspensions containing 2 x 10^6 spores ml^{-1} of the fungus, hatch did not exceed 16.5 per cent and the hatched out larvae died soon. Nene (1973) reported the same fungus as a pathogen of adults of *Bemisia tabaci.*

Ramisch (1976) recorded mortality of *L. decemlineata* by *P. farinosus.* Another isolate of *P. farinosus* was found to infect the larvae of *Sylepta derogata, Antoba olivacea, Diacrisia obliqua, Margaronia indica, Plusia peponis, Hymenia recurvalis, Psara basalis, Nephantis serinopa* and adults of *D.cingulatus, N. lugens* and *Pentalonia nigronervosa* (Kuruvilla and Jacob, 1980). Gopalakrishnan *et al.* (1999) proved the efficacy of *P. farinosus* against *P. xylostella*, both under glasshouse and field conditions.

Ibrahim and Low (1993) noticed significant reduction in the larval population of *P. xylostella* by the application of spore suspension of *Paecilomyces fumosoroseus* and *B. bassiana. P. fumosoroseus* was reported as one of the microbial agent against *Bemisia* spp. and other white flies under glasshouse conditions (Lacey and Fransen, 1994).

The fungus was lethal to third instar larvae of *S. litura, H. armigera* and adults of *Aphis gossypii* at a concentration of 1 x 10^8 conidia ml^{-1} (Hassani *et al.*, 2000) and caused significant reduction in the population of yellow mite *Polyphagotarsonemus latus* (Ramarethinam *et al.*, 2001).

2.1.6 *Entomophthora* spp.

Strawinski (1930) first reported frequent epizootics caused by entomogenous fungus *Entomophthora sphaerosperma* on *Pieris brassicae* in Europe, and observed that the fungus regulated the outbreak of the pest. The same fungus was identified by Shaw (1959) as a pathogen of *Plutella maculipennis* in Britain. *Entomophthora coronata* was reported as a parasite of *M. persicae, Rhophalosiphum erysimi* and *Brevicoryne brassicae* feeding on *Brassica* spp and radish under very

high humid conditions in India (Ramaseshiah, 1967) while *Entomophthora blunckii* was reported from cabbage moth, *P.maculipennis* (Zimmermann, 1978).

Hussey and Tinsley (1981) reported that conidial suspensions of the fungus *Entomophthora aphidis* killed *A. gossypii, Aphis pomi, Sitobion avenae* and *M. persicae.* The resting spores of *Entomophthora thaxteriana* killed the field population of pea aphid *Achanthosiphon pisum* (Varonina *et al.*, 1987).

2.1.7 *Verticillium* spp.

Two isolates of the fungus *Verticillium lecanii* were reported on the whitefly, *T. vaporariorum* and aphid *A. gossypii* in glasshouse conditions (Hall, 1982). This fungus caused mortality of aphid *M. persicae* under field conditions also. Grunberg *et al.* (1988) reported that *V. lecanii* effectively managed the aphids *M. persicae, A. gossypii* and *Aphis fabae* under greenhouse conditions.

Meade and Byrne (1991) reported mortality of all the three nymphal stages of *Bemisia tabaci* by the fungus *V. lecanii* and Gindin *et al.* (2000) reported the pathogenicity of the fungus on eggs, third instar nymphs, pupae and adults of *B. argentifolii.*

2.2 BIOASSAY OF ENTOMOPATHOGENIC FUNGI

Badilla and Alves (1991) conducted bioassays under *in vitro* conditions using four dosages of *B. bassiana* against *Sphenophorus levis.* They obtained an LC_{50} value of 8.8×10^9 conidia ml^{-1} while Dorschner *et al.* (1991) recorded an LC_{50} of 1.37×10^5 conidia ml^{-1} when *B. bassiana* was used against the hop aphid, *Phorodon humuli.*

Ninety five per cent mortality of larvae of *Ostrinia fumacata* was obtained when *B. bassiana* at 50×10^9 spores ml^{-1} was sprayed on them (Zhang *et al.*, 1992). Brenes and Carballo (1994) determined the LC_{50} and LC_{90} values of A_4 strain of *B. bassiana* against *C. sordidus* as 7.89 x

10^7 and 2.67 x 10^9 spores ml^{-1} respectively. They obtained the LT_{50} value between 6.3 and 10 days.

Negasi *et al.* (1998) reported that the isolate L_{3009} of *B. bassiana* was pathogenic to first instars and adults of *B. argentifolii* with LC_{50} and LC_{90} values of 1.3 x 10^6 and 1.0 x 10^7 conidia ml^{-1} respectively. Adults of shot hole borer of tea, when sprayed with *B. bassiana* spore suspensions (10^5, 10^6, 10^7 and 10^8 spores ml^{-1}) under *in vitro* conditions, caused 100 per cent mortality within 5 – 6 days at 10^7 and 10^8 spores ml^{-1} (Selvasundaram and Muraleedharan, 2000).

Khan and Gangapersad (2001) reported LC_{50} value of 4.5 x 10^7 spores ml^{-1} of *B. bassiana* against banana bore weevil, *C. sordidus*. Ignoffo *et al.* (1983) conducted a leaf surface treatment bioassay under field conditions for determining the activity of conidia of *B. bassiana* against *L. decemlineata* and found out that 200, 16.25 and 0.75 kg conidia ha^{-1} caused 99, 90 and 50 per cent mortality of the larvae.

Hareendranath (1989) conducted bioassay using *F. pallidoroseum* on *A. craccivora* under laboratory conditions and calculated LC_{50} value as 3.048 x 10^6 spores ml^{-1}.

Blastospores of *V. lacanii* at 2.5 x 10^{13} spores l^{-1} gave complete control of chrysanthemum aphids *A. gossypii* under glasshouse conditions (Sopp *et al.*, 1989).

Chen *et al.* (1995) observed 100 per cent mortality of *Holotrichia paralella* when an isolate of *M. anisopliae* was applied to soil at a concentration of 2.5 x 10^6 spores g^{-1} under *in vitro* conditions and another isolate of the same fungus showed 54 – 100 per cent mortality of *Megalothrips sjostedti* (Ekesi *et al.*, 1998). They obtained the LC_{50} and LT_{50} of the fungus as 1.3 x 10^6 conidia ml^{-1} and 2.4 days respectively. Vanninen *et al.* (1999) obtained 40 – 50 per cent mortality of third instar larvae of cabbage root fly *D. floralis* when exposed to 1.5 x 10^{10} spores ml^{-1} of *M. anisopliae*.

In laboratory bioassays the fungal spore concentration of 40 x 10^8 spores ml^{-1} of *M. anisopliae* caused 92 and 90 per cent mortality of 3 – 5 day old and 5 – 8 day old cabbage semilooper (Wickramatileke *et al.*, 2000). They stated that a concentration of 86 x 10^7 spores ml^{-1} was optimum under laboratory conditions. LC_{50} values of 5.13 x 10^7 and 4.92 x 10^8 spores ml^{-1} of *M. anisopliae* and *Metarhizium flavoviridae* were obtained against banana weevil, *C. sordidus* (Khan and Gangapersad, 2001).

A spore concentration of 1x 10^8 conidia ml^{-1} of *P. fumosoroseus* caused 76.20, 59.60 and 95.80 per cent mortality of third instar larvae of *S. litura, H. armigera* and adults of *A. gossypii* respectively (Hassani *et al.*, 2000).

2.3 MASS MULTIPLICATION OF ENTOMOPATHOGENIC FUNGI

Ease and low cost of production and application, are among the principal characteristics of a desirable pathogen. Most of the fungal products used in biological control are produced *in vitro*. The scale of production ranges from small-scale agar based production to industrial scale production (Pereira and Roberts, 1990).

Ferron (1981) reported solid-substrate fermentation based on cereal grains such as rice or static liquid culture as suitable for fungal pathogens while Jenkins and Prior (1993) recommended deep tank liquid fermentation to produce mycelial products, blastospores and submerged conidia.

2.3.1 Solid Substrates

Growth and sporulation of *B. bassiana* infecting *P. xylostella* was maximum on rice among the different loose-solid media tested by Ibrahim and Low (1993). Similar observation was also made by Vilas *et al.* (1996). Puzari *et al.* (1997) reported a mixture of rice hull, saw dust and rice bran in the ratio 75 : 25 : 100 to be an ideal substrate for mass production of the fungus.

Chaudhuri *et al.* (2001) tested various substrates including grains and agricultural byproducts for mass multiplication of *B. bassiana* and based on biomass production, conidial count, radial growth and viability of conidia, sorghum grains was found to be the best substrates to support growth and sporulation of the fungus. According to Haraprasad *et al.* (2001) *B. bassiana* grew well on rice bran (fine), rice bran (coarse) and wheat bran yielding a maximum sporulation.

Beevi (1979) reported sorghum and bajra to be the most suitable food source for mass multiplication of *F. moniliformae* var.*subglutinans* pathogenic to epilachna beetle. Broken maize grains and black gram husk or red gram husk at 4:1 (w/w) ratio was found to be an ideal substrate for mass multiplication of *F. semitectum* infecting *M. persicae* (Nagalingam, 1983).

Mathai *et al.* (1988) observed maximum spore count of *F. pallidoroseum* when it was grown on wheat bran or rice bran plus tapioca bits while the growth was very poor on vegetable waste and on tapioca stem peelings.

According to Faizal (1992) growth of *F. pallidoroseum* was quick on wheat bran compared to other substrates like rice bran and combination of bran and straw bits. However, Rejirani (2001) obtained good growth and sporulation of the same fungus on rice bran and gingelly oil cake.

Daoust and Roberts (1983) observed rice medium to be a better and cheaper substrate than the more complex mycological media for the production of conidia of *M. anisopliae*. Alvarez *et al.* (1997), Narvaez *et al.* (1997) and Jenkins *et al.* (1998) also reported sterile humid rice as the best substrate for growing *M. anisopliae*.

Easwaramoorthy and Jayaraj (1978) obtained good sporulation of *Cephalosporium lecanii* when it was grown on moist sterile sorghum grains for three weeks.

2.3.2 Liquid Substrates

Batista *et al.*(1989) observed better conidial production of *B. bassiana* in bran broth compared to rice and potato broth. Ibrahim and Low (1993) identified coconut water as the best liquid medium for production of *B. bassiana*.

Manisegarame and Letchoumanane (1996) tried coconut water, parboiled rice water, parboiled rice gruel, raw rice water and raw rice gruel for mass production of *F. pallidoroseum* and observed maximum biomass production in coconut water compared to other substrates. The beneficial effects of coconut water was also reported by Rejirani (2001).

Danger *et al.* (1991) used coconut water from copra making industry for mass production of *M. anisopliae* against rhinoceros beetle, *Oryctes rhinoceros*.

2.4 STORAGE OF ENTOMOPATHOGENIC FUNGI

Shelf life is an important criterion to be taken into consideration while developing a biopesticide. Effect of substrate on the retention of conidial viability and virulence of an entomopathogenic fungus after prolonged storage should be assessed for selection of ideal substrate for growth of the fungi.

Walstad *et al.* (1970) noticed that the survival of spores of the muscardine fungi over extended periods of time was affected by the storage temperature. They observed that the spores of *B. bassiana* and *M. anisopliae* remained viable for twelve months at 8^0C, but lost the virulence after 0.5 and 2.5 months at 21^0C.

Blachere *et al.* (1973) observed 85 per cent and 100 per cent reduction in the survival of *Beauveria brongniartii* when it was stored for four and seven weeks respectively at 23^0C. However, when it was stored at 4^0C the percentage of spores survived was 16 even after eight months.

Aregger (1992) reported 98 per cent viability of conidia of *B. brongniartii* for two years when stored at 2^0C and the survival of conidia of *Beauveria* was maximum when it was stored in water at 15 – 25^0C. The spores of *B. bassiana* formulated as wettable powder retained more than 85 per cent spore germination even after eight months when stored under refrigerated condition. The pathogenicity of the fungus was maintained even after one year at room temperature (Zhang et al., 1992).

Spores of *F. pallidoroseum* isolated from *A. craccivora* and formulated in water, talc and diatomaceous earth retained 75 per cent viability till four days of storage and thereafter a significant reduction was noticed in the virulence (Faizal, 1992).

Sunitha (1997) observed that the shelf life of *F. pallidoroseum* spores could be doubled by storing the spores in diatomaceous earth under refrigeration, compared to room temperature. The virulence was reduced to 50 per cent at the end of ten months when stored at room temperature while at refrigeration 91.4 per cent of spores retained their virulence.

Rejirani (2001) identified semidry cadaver, diatomaceous earth, fine charcoal powder and leaf mould to be the ideal carriers to conserve viability and virulence of the spores of *F. pallidoroseum*. Storage under refrigeration was found to improve the spore qualities when compared to storage at room temperature.

Entomogenous fungus *Paecilomyces cicada* isolated from cicadid *Platylorria pieli* retained its pathogenicity after storage for one year at room temperature (Chen *et al.*, 1990). But only few blastospores survived after 24 h of storage on silica gel, sand or diatomaceous earth at 20 – 23^0C in the case of *P. fumosoroseus*.

Moore *et al.* (1995) observed 42 per cent germination of *M. flavoviridae* after four to five months of storage as powder at 25 – 37^0C while the germination percentage increased to 97 at 10 –12^0C.

2.5 EFFECT OF PESTICIDES ON GROWTH AND SPORULATION OF ENTOMOPATHOGENIC FUNGI

One of the most promising aspects of microbial control agent of insect pest is its integration with other pest control measures, particularly the chemical method (Urs *et al.*, 1967). Several pesticides have been reported to have varying effects on entomopathogenic fungi from inhibitory to stimulatory.

2.5.1 Insecticides

Anderson and Roberts (1983) found that generally the wettable powder and flowable formulations cause no inhibition, and often increase the colony counts whereas emulsifiable concentrate formulations frequently inhibit *B. bassiana* germination.

Carbaryl at 0.1, 1, 10, 100 and 1000 ppm inhibited the germination of conidia of the entomogenous fungi *B. bassiana*, *M. anisopliae* and *Hirsutella citriformis* (Aguda *et al.*, 1988). Anderson *et al.* (1989) reported that abamectin, triflumuron, thuringiensin and carbaryl demonstrated no significant inhibition of colony growth of the fungus, *B. bassiana*.

Moniliale fungi like *B. bassiana, P. farinosus* and *V. lecanii* showed better growth on the medium containing 25 per cent diflubenzuron at the two lower doses while, at ten times the field dose, growth was inhibited (Sapieha and Mietkiewski, 1992).

Faizal and Mathai (1997) reported complete inhibition of growth of *F. pallidoroseum* by HCH. Monocrotophos and mercaptothion produced less inhibition on growth and allow fairly good sporulation. Fenthion showed least inhibition on sporulation of the fungus but caused inhibition of growth. Rejirani (2001) reported that quinalphos at 0.05 per cent concentration did not suppress the growth and sporulation of *F. pallidoroseum* completely.

Ignoffo *et al.* (1975) reported that pesticides recommended for use in soybeans suppressed the growth of *Nomuraea rileyi* at one tenth of the recommended concentration. Gopalakrishnan and Mohan (2000) reported that insecticides monocrotophos, phosphamidon and dimethoate were comparatively safe to the entomopathogenic fungus *N. rileyi* at lower as well as higher concentrations, but quinalphos, carbaryl, endosulfan and fenvalerate were safe at lower concentrations (0.005, 0.025, 0.05 %) but were highly detrimental to the fungus at higher concentrations (0.15 and 0.75 %).

Four concentrations (50, 100, 200 and 500 ppm) of water dispersible formulation of imidacloprid significantly reduced the germination of primary conidia of *Neozygites floridana* but 100 ppm solution increased the conidial germination in *Hirsutella thompsonii* (Dara and Hountondji, 2001).

Eayre *et al.* (1990) also reported better germination of *Hirsutella rhossiliensis* by imadacloprid.

2.5.2 Fungicides

Todorova *et al.* (1998) studied the effect of six fungicides (chlorothalonil, maneb, thiophanate-methyl, mancozeb, metalaxyl + mancozeb and zineb) on *B. bassiana in vitro*. All the six fungicides inhibited the mycelial growth and sporulation.

Faizal and Mathai (1997) observed complete inhibition of sporulation of *F. pallidoroseum* by copper oxychloride, mancozeb, captofol, captan and zineb. Captan and zineb inhibited the vegetative growth of the fungus.

Inhibition of conidial germination of *Neozygites floridana* by benomyl was reported by Brandenberg and Kennedy (1983). *Hirsutella citriformis* was susceptible to benomyl at 10 and 100 ppm (Aguda *et al.*, 1988).

2.5.3 Botanical Pesticides

Gupta *et al.* (1999) observed that commercial neem products like Neem Gold, Neemark and neemcake-water-extract had inhibitory effect of mycelial growth of *B. bassiana* in direct proportion to the concentration of chemical or extract. They also observed that margocide, nimbicidine and neem cake-alcohol-extract had no effect on growth of *B. bassiana* and neem-leaf-water and neem-leaf-alcohol extracts, Field Marshal and Achook (Neem products) stimulated the fungal growth in direct relation to chemical concentration.

Rejirani (2001) reported that seed oil emulsions of neem and marotti at 10 per cent highly inhibited the sporulating ability of the fungus *F.pallidoroseum*, but leaf extracts of neem, bougainvillea and hyptis were found to enhance sporulation.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

Experiment on the "Effect of entomopathogenic fungi on sucking pests and leaf feeders of vegetables under *in vitro* conditions" was conducted in the insect pathology laboratory, College of Agriculture, Vellayani during 2000 – 2002.

3.1 PATHOGENICITY TESTS OF ENTOMOPATHOGENIC FUNGI ON VARIOUS VEGETABLE PESTS

Experiments were conducted under *in vitro* conditions to study the pathogenicity of different entomopathogenic fungi on various sucking pests and leaf feeders of vegetables.

Table 1. Entomopathogenic fungi used for the study

Fungus	Source
1.*Metarhizium anisopliae*.(Metch)	Central Plantation Crop Research Institute, Kayamkulam, Kerala
2.*Beauveria bassiana* (Balsamo) Vuillemin	Department of Entomology, Tamil Nadu Agricultural University, Coimbatore
3.*Beauveria brongniartii* (Sacc.) Petch	Sugarcane Breeding Institute, Coimbatore
4.*Paecilomyces fumosoroseus* (Wize) Brown	Department of Entomology, Tamil Nadu Agricultural University, Coimbatore
5.*Paecilomyces lilacinus* (Thom.) Samson	Indian Type Culture Centre, Indian Agricultural Research Centre, New Delhi
6.*Rhizopus oryzae* Went and Gerlings	Insect Pathology Laboratory, College of Agriculture, Vellayani
7.*Fusarium pallidoroseum* (Cooke) Sacc.	Insect Pathology Laboratory, College of Agriculture, Vellayani

3.2 TEST INSECTS

3.2.1 Sucking Pests

Ten Hemipteran pests were collected from the Instructional Farm of the College of Agriculture, Vellayani and reared in the laboratory

using their respective host plants. These insects were used for testing the pathogenicity of entomopathogenic fungi listed under Table 1.

Table 2. List of sucking pests used in pathogenicity tests.

Insect species	Host plant
1. *Aphis gossypii* Gl.	Brinjal (*Solanum melongena*)
2. *Coccidohystrix insolitus* (Gr)	Brinjal (*Solanum melongena*)
3. *Bemisia tabaci* G	Brinjal (*Solanum melongena*)
4. *Aphis malvae* Koch.	Bhindi (*Abelmoschus esculentus*)
5. *Amrasca devastens* (Ishida)	Bhindi (*Abelmoschus esculentus*)
6. *Aleurodicus dispersus* Russel	Bhindi (*Abelmoschus esculentus*)
7. *Dysdercus cingulatus* F.	Bhindi (*Abelmoschus esculentus*)
8. *Myzus persicae* (Sulz.)	Chilli (*Capsicum annuum*)
9. *Aphis craccivora* Koch.	Cowpea (*Vigna ungiculata*)
10. *Riptortus pedestris* Fb.	Cowpea (*Vigna ungiculata*)

3.2.2 Leaf Feeders

The immature stages of five Lepidopteran and one Coleopteran pests were collected from the Instructional Farm of College of Agriculture, Vellayani and used for testing the pathogenicity of the entomopathogenic fungi listed under 3.1.

Table 3. List of leaf feeders used in pathogenicity tests

Pest	Host plant
Lepidopteran pests	
Sylepta derogata Fb.	Bhindi (*Abelmoschus esculentus*)
Spodoptera litura Fb.	Bhindi (*Abelmoschus esculentus*)
Diaphania indica Saund.	Snakegourd (*Trichosanthes anguina*)
Anadevidia peponis Fb.	Snakegourd (*Trichosanthes anguina*)
Pericallia ricini Fb.	Drumstick (*Moringa oleifera*)
Coleopteran pest	
Henosepilachna vigintioctopunctata (Fab.)	Brinjal (*Solanum melongena*)

Pathogenicity of the entomopathogenic fungi listed under 3.1 was tested on the leaf feeders and sucking insects mentioned above. The entomopathogenic fungi were grown on Potato Dextrose Agar slants. Spores were harvested from ten-day-old culture. Sterile distilled water (5 ml each) was added to these cultures and shaken thoroughly in order to prepare a spore suspension and this concentrated spore suspension was used for inoculating the insects.

The spore suspension was sprayed on the insects using an atomizer. Insects sprayed with sterile distilled water served as the control. Treated insects were transferred to glass jars and reared on respective host plants. For maintaining high humidity inside the chamber, a sheet of moist filter paper was placed at the bottom of the container. The inoculated insects were examined constantly for the development of disease symptoms. Mortality due to fungal infections was identified by the stiff and mummified appearance of the cadaver. Pathogenicity of the fungus was confirmed by proving Koch's postulates. Only those fungi, which were pathogenic to insects, were used for further studies

3.3. MAINTENANCE OF FUNGAL CULTURE

The fungal cultures were maintained by periodic transfer on potato dextrose agar slants. Virulence of the fungi was maintained by passing them periodically through their respective hosts and reisolating the fresh cultures.

3.4 BIOASSAY OF ENTOMOPTHOGENIC FUNGI

A preliminary study was conducted to determine the doses of fungi used for bioassay against different pests. The concentrations giving mortality percentage in the range of 15 – 85 were selected. Materials used for bioassay of different fungi are given below:

Table 4. Fungus, doses and target pests used for bioassay

Fungus	Test insect and stage	Concentration of spray fluid (spores / ml)
1. *Beauveria bassiana*	*Sylepta derogata* (Fourth instar larvae)	1.25×10^8 2.50×10^8 5.00×10^8 1.00×10^9 2.00×10^9
	Spodoptera litura (Third instar larvae)	1.25×10^8 2.50×10^8 5.00×10^8 1.00×10^9 2.00×10^9
	Pericallia ricini (Third instar larvae)	1.25×10^5 2.50×10^5 5.00×10^5 1.00×10^6 2.00×10^6
2. *Fusarium pallidoroseum*	*Aphis craccivora* (Wingless adults)	0.437×10^6 0.875×10^6 1.75×10^6 3.50×10^6 7.00×10^6
	Myzus persicae (Wingless adults)	0.437×10^6 0.875×10^6 1.75×10^6 3.50×10^6 7.00×10^6
3. *Rhizopus oryzae*	*Dysdercus cingulatus* (Third instar nymphs)	1.25×10^6 2.50×10^6 5.00×10^6 1.00×10^7 2.00×10^7

3.4.1 Preparation of Spray Fluid

For preparing different spore suspensions of the test fungi for bioassay, stock suspension of highest spore concentration was prepared. For this seven mm fungal discs from well sporulating fungal cultures on PDA grown in petridishes were used. The discs were shaken well in 10 ml water blank and spore count was estimated using a

haemocytometer. Spore concentrations were adjusted either by adding additional fungal discs or sterile water. Serial dilution was done to prepare the suspensions of lower concentrations.

3.4.2 Bioassay

The test insects of uniform age and size taken from laboratory culture were selected for the bioassay. Insects were sprayed with different spore concentrations of fungus (Table 4) using an atomizer. Insects sprayed with sterile water served as control. Replications were maintained in each case. Observations were recorded on insect mortality at daily intervals till pupation of Lepidopteran pests and till 100 per cent mortality or the subsequent moulting in the case of Hemipteran pests. The dosage mortality data obtained were subjected to Probit Analysis (Finney, 1952) and LC_{50} value of each fungus was calculated.

3.5 MASS MULTIPLICATION OF ENTOMOPATHOGENIC FUNGI

In order to find out the best medium for mass multiplication of the fungi, locally available solid and liquid substrates were used as food sources.

3.5.1 Substrates Used for Mass Multiplication

The following substrates were used for mass multiplication of *Beauveria bassiana*, *Fusarium pallidoroseum* and *Rhizopus oryzae*.

1. Rice bran
2. Gingelly oil cake
3. Coconut water
4. Starch solution (Rice gruel)
5. Water

3.5.2 Mass Multiplication on Solid Substrates

Thirty gram each of the substrates were taken in 250 ml conical flasks and enough quantity of water was added to make them just wet.

Gingelly oil cake was powdered in a grinder before using. The flasks were then plugged with cotton and autoclaved at 1.1 kg cm^{-2} for twenty minutes in an autoclave. The sterilized substrates were then artificially inoculated with seven ml fungal discs from ten-day-old fungal cultures under aseptic condition. The flasks were shaken well for even distribution of the fungus in the medium. They were incubated at room temperature. Growth of the fungus was observed by visual comparison. For estimating the spore load in each medium, 150 ml of sterile distilled water was added to each of the conical flasks and shaken vigorously for two minutes. The suspensions obtained from the replications were taken separately and filtered through a muslin cloth. From this one drop of the suspension was used for estimating the spore count using a haemocytometer. The spore count of *B. bassiana*, *F. pallidoroseum* and *R. oryzae* were estimated on eleven, seven and six days after inoculation respectively.

3.5.3 Liquid Substrates

Coconut water, starch solution and plain water were used as liquid substrates for growing the fungi. For preparing the starch solution 200 g of parboiled rice was cooked in two litres of water. After proper cooking the water was decanted and cooled. This was used for growing the fungus.

The liquid substrates (125 ml each) were taken separately in 250 ml conical flasks, plugged with cotton and sterilized at 1.1 kg cm^{-2} for 20 minutes in an autoclave. After sterilization, they were inoculated with ten day old culture bits of the fungus and incubated at room temperature. The intensity of sporulation was estimated using a haemocytometer.

3.6 SHELF LIFE STUDIES

Viability and virulence of the fungi grown on the substrates *viz.*, rice bran, gingelly oil cake, coconut water and starch solution upon

storage at room temperature (21.5 – 32.5^{0}C) was studied for a period of six months at monthly intervals.

3.6.1 Assessment of Viability of the Fungus

Spore concentration of the fungus in the medium at monthly intervals was estimated by suspending one gram of the substrate in 50 ml sterile water in 250 ml conical flasks. In the case of liquid media, one ml of the medium was pipetted out into 50 ml sterile water in 250 ml conical flask. The liquid medium was shaken for one minute, before taking the sample. The suspension was shaken thoroughly for two minutes and sieved through a muslin cloth. This suspension was used for the estimation of spore concentration using a haemocytometer. To assess the viability of the inoculum, one drop of this suspension was placed on PDA in a 9 cm petridish and growth of the fungus was recorded by measuring the colony diameter at the end of 10 days in the case of *B. bassiana* and *F. pallidoroseum* and three days in the case of *R. oryzae*.

3.6.2 Assessment of Virulence of the Fungus

Virulence of the fungus grown in different substrates was studied at monthly intervals by testing their pathogenicity. For this, spore suspension from different substrates were standardized to a concentration of 2 x 10^{9}, 7x 10^{6} and 2 x 10^{7} spores/ml of *B. bassiana, F. pallidoroseum* and *R. oryzae* respectively. Fourth instar larvae of *Sylepta derogata*, wingless adults of *Aphis craccivora* and third instar nymphs of *Dysdercus cingulatus* were used respectively for testing the virulence of *B. bassiana, F. pallidoroseum* and *R. oryzae*. The test insects were sprayed with the fungal suspension and were observed for symptom development at 24 h interval. Insects sprayed with sterile water served as control. The cumulative per cent mortality was analysed statistically.

3.7 COMPATIBILITY OF ENTOMOPATHOGENIC FUNGI WITH PESTICIDES

This experiment was carried out to find out whether the pesticides commonly used against vegetable pests have any detrimental effect on the growth and sporulation of *B. bassiana*, *F. pallidoroseum* and *R. oryzae*. The pesticides and the concentration used for this experiment are given below:

1. Chlorpyriphos 0.05 % (Radar 20 EC)
2. Malathion 0.1 % (Malathion 50 EC)
3. Quinalphos 0.05 % (Ekalux 25 EC)
4. Dimethoate 0.05 % (Rogar 30 EC)
5. Mancozeb 0.2 % (Indofil-M-45)
6. Neem oil emulsion 2 %

3.7.1 Growth and Sporulation of the Fungus on Poisoned Media

Poison Food Technique (Zentmeyer, 1955) was used to find out the rate of inhibition of the growth of entomopathogenic fungi in the media incorporated with different pesticides. Measured quantities of pesticides were mixed with 100 ml each of sterilized potato dextrose agar medium taken in 250 ml conical flasks when it was in a molten stage (45^0C). Flasks were shaken thoroughly and the media were poured into sterile petriplates of 9 cm diameter and allowed to solidify.

Circular discs of 7 mm from the outer edges of fresh fungal cultures were transferred to the centre of these plates using a sterile cork borer. Media without pesticide and inoculated with the fungus was served as control. The inoculated plates were incubated at room temperature. Radial growth of the fungus was measured daily until the fungal growth in the control covered the entire plate. To assess the sporulation, five circular discs of 7 mm were taken from the culture from different areas of the plate. These discs were mixed in 10 ml of

sterile water and shaken for two minutes. The spore concentration of this suspension was estimated using a haemocytometer.

RESULTS

4. RESULTS

4.1 PATHOGENICITY TESTS

Ten species of sucking pests and six species of leaf feeders were used for the study. The test insects sprayed with different entomopathogenic fungi were kept under observation for the development of disease symptoms. Out of the seven fungal pathogens tested, Koch's postulates could be proved only with three isolates *viz.*, *Beauveria bassiana* against *Sylepta derogata*, *Spodoptera litura* and *Pericallia ricini*, *Fusarium pallidoroseum* against *Aphis craccivora* and *Myzus persicae* and *Rhizopus oryzae* against *Dysdercus cingulatus* (Table 5).

The systematic position of the insects susceptible to the fungal pathogens are given in Table 6.

4.1.1 Mycoses of Test Insects Infected With the Fungus

4.1.1.1 Beauveria bassiana

The three lepidopteran insects susceptible to *B. bassiana* were inoculated artificially and the detailed symptom development was worked out. All the insects developed almost similar symptoms after inoculating with *B. bassiana*. The insects did not show visible symptoms for two days. However, in *P. ricini* the food uptake was reduced considerably during the first 24 hours itself. The insects were sluggish in their movement. The infected larvae of *P. ricini* died on the second day onwards. Larvae of *S. derogata* and *S. litura* became sluggish and inactive only after three days.

The infected larvae failed to moult and death occurred after four to five days after inoculation. The cadavers of all the three groups of insects appeared stiff and mummified. White mycelial growth of the fungus appeared all over the cadaver within three to four days after death. When the dead insects were kept in moist chamber, mycelial

Table 5. Pathogenicity of entomopathogenic fungi on various vegetable pests

Sl. No.	Test insects	*B. bassiana*	*B. brongniartii*	*M. anisopliae*	*F. pallidoroseum*	*P. fumosoroseus*	*P. lilacinus*	*R. oryzae*
	Sucking pests							
1.	*Aphis gossypii*	Negative	Negative	Negative	Negative	Negative	Negative	Negative
2.	*Aphis malvae*	Negative	Negative	Negative	Negative	Negative	Negative	Negative
3.	*Amrasca devastens*	Negative	Negative	Negative	Negative	Negative	Negative	Negative
4.	*Aleurodicus dispersus*	Negative	Negative	Negative	Negative	Negative	Negative	Negative
5.	*Dysdercus cingulatus*	Negative	Negative	Negative	Negative	Negative	Negative	**Positive**
6.	*Bemisia tabaci*	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7.	*Coccidohystrix insolitus*	Negative	Negative	Negative	Negative	Negative	Negative	Negative
8.	*Myzus persicae*	Negative	Negative	Negative	**Positive**	Negative	Negative	Negative
9.	*Aphis craccivora*	Negative	Negative	Negative	**Positive**	Negative	Negative	Negative
10.	*Reptortus pedestris*	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Leaf feeders								
1.	*Henosepilachna vignitioctopunctata*	Negative	Negative	Negative	Negative	Negative	Negative	Negative
2.	*Sylepta derogata*	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative
3.	*Pericallia ricini*	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative
4.	*Spodoptera litura*	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative
5.	*Diaphania indica*	Negative	Negative	Negative	Negative	Negative	Negative	Negative
6.	*Anadevidia peponis*	Negative	Negative	Negative	Negative	Negative	Negative	Negative

Table 6. Target pests of selected entomopathogenic fungi

Fungus	Target insect pest		
	Species	Family	Order
Beauveria bassiana	*Sylepta derogata*	Pyralidae	Lepidoptera
	Spodoptera litura	Noctuidae	Lepidoptera
	Pericallia ricini	Arctiidae	Lepidoptera
Fusarium pallidoroseum	*Aphis craccivora*	Aphididae	Hemiptera
	Myzus persicae	Aphididae	Hemiptera
Rhizopus oryzae	*Dysdercus cingulatus*	Pyrrhocoridae	Hemiptera

growth appeared within two days after the death. Within one week, the whole surface of the dead insect was covered with white coloured powdery spores of the fungus (Plate 1, 2 and 3).

*4.1.1.2 **Fusarium pallidoroseum***

F.pallidoroseum was infective only on the aphids, *M. persicae* and *A. craccivora.* The aphids infected with *F. pallidoroseum* turned pale, sluggish and later turned brown in colour, and died within two to three days after infection. The cadaver was hard and mummified and seen firmly adhered to the host plant surface. Growth of the mycelium over the cadaver was observed four to five days after infection (Plate 4 and 5).

*4.1.1.3 **Rhizopus oryzae***

The red cotton bugs became sluggish within three to five days and death occurred within five to six days after inoculation. The cadaver was hard and mummified. External mycelial growth occurred on the cadaver from the third day after death. The mycelia was off- white in colour and sporulation occurred within four to five days (Plate 6).

4.2 BIOASSAY OF ENTOMOPATHOGENIC FUNGI

4.2.1 ***Beauveria bassiana***

Five geometric proportions of concentration of spores of *B.bassiana* was used in the bioassay of the fungus against three lepidopteran pests *viz.*, *S. derogata*, *S. litura* and *P. ricini* and LC_{50} values were calculated.

*4.2.1.1 **Sylepta derogata***

Death of the 4th instar larvae of *S. derogata* inoculated with different spore concentrations of *B. bassiana* was noticed from the 4th day after inoculation. Mortality rate of the insects increased with increase in the spore concentration. The mortality on the 8th day after inoculation (Table 7) ranged from 10 to 73.33 per cent as the

Larvae

Pupae

Plate 1. *S.derogata* infected with *B.bassiana*

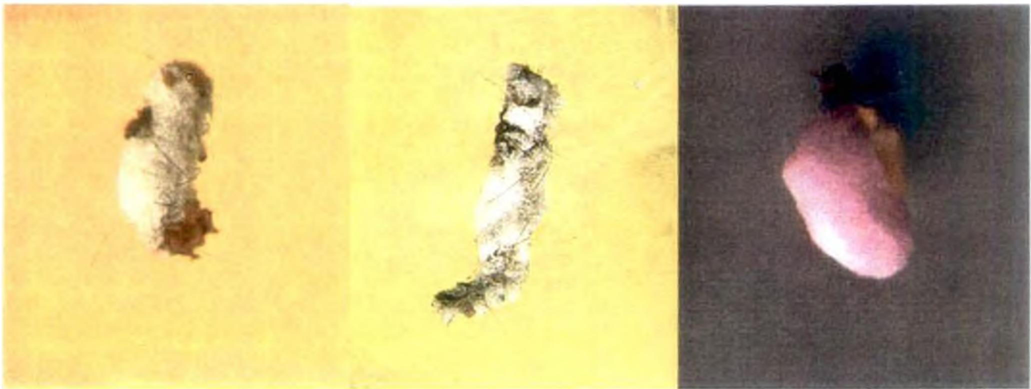

Plate 2. *P.ricini* larvae infected with *B.bassiana*

Plate.3. *S.litura* larvae infected with *B.bassiana*

Plate 4. *A.craccivora* infected with *F.pallidoroseum*

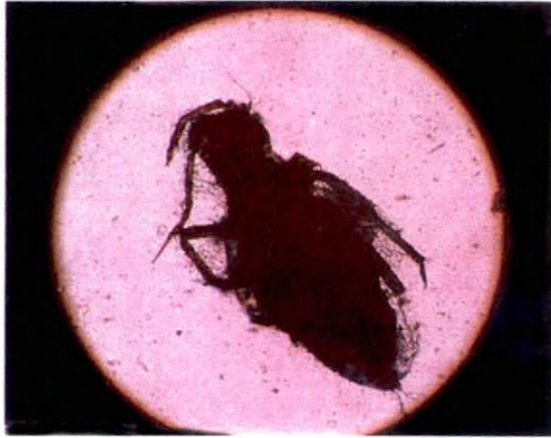

Plate 5. *M.persicae* infected with *F.pallidoroseum*

Plate 6. *D.cingulatus* infected with *R.oryzae*

concentration increased from 1.25 x 10^8 to 2 x 10^9 spores ml^{-1}. On the 9th day, the mortality increased only in those treatments where insects were sprayed with 1 x 10^9 and 2 x 10^9 spores ml^{-1}, giving 93.33 and 96.66 per cent mortality respectively. Eventhough all the larvae which were not killed, moulted and entered into pupation, none of them emerged as adults.

Results of probit analysis of dose-mortality responses of the larvae on 8th and 9th day after inoculation obtained the LC_{50} values as 7.87 x 10^8 and 5.75 x 10^8 spores ml^{-1} respectively (Table 8). The fiducial limit calculated on the 8th day indicated that for 50 per cent mortality, the spore concentration of the inoculum should be in the range of 7.09 x 10^8 to 8.66 x 10^8 per ml and the corresponding figures for the 9th day falls within the range 5.38 x 10^8 to 6.13 x 10^8 spores ml^{-1}.

4.2.1.2 Spodoptera litura

Third instar larvae of *S. litura* were used for bioassay of *B. bassiana*. The mortality of the larvae was recorded on fourth day of inoculation and it ranged from 1.66 to 55 per cent as the spore concentration increased from 1.25 x 10^8 to 2 x 10^9 spores ml^{-1}. Death of the larvae occurred only upto 6th day of inoculation and the remaining uninfected larvae moulted, pupated and emerged as adults. On the 6th day the mortality increased from five per cent to 95 per cent with the increase in the spore concentration (Table 9). Based on the data, fiducial limits and LC_{50} values were calculated and from these it can be extrapolated that 50 per cent mortality of the larvae is possible on the fourth day if the spore concentration is between 14.5 x 10^8 and 18.3 x 10^8 spores ml^{-1}. Similarly, to get 50 per cent death on the 6th day, the spore concentration required was between 6.51 x 10^8 and 7.47 x 10^8 spores ml^{-1} (Table 10).

Table 7. Cumulative per cent mortality of fourth instar larvae of *S. derogata* treated with different spore concentrations of *B. bassiana*

Concentration, spores ml^{-1}	Cumulative percentage mortality at days after treatment					
	4	5	6	7	8	9
1.25×10^8	0	1.66	6.66	10.00	10.00	10.00
2.50×10^8	0	2.33	10.00	13.33	13.33	13.33
5.00×10^8	6.66	10.00	13.33	26.66	30.00	30.00
1.00×10^9	10.00	13.33	20.00	36.66	76.60	93.33
2.00×10^9	10.00	13.33	20.00	46.66	73.33	96.66
Control	0	1.66	2.33	2.33	2.33	2.33

Table 8. Probit analysis of dose-mortality responses of fourth instar larvae of *S. derogata* to varying doses of *B. bassiana*

Days after treatment	χ^2	Regression equation	LC_{50} spores/ml $\times 10^8$	Fiducial limit $\times 10^8$
8	11.2990	Y=3.1087 + 2.1099x	7.87	7.09-8.66
9	16.1950	Y=1.9800 + 3.9727x	5.75	5.38-6.13

Table 9. Cumulative percentage mortality of third instar larvae of *S. litura* treated with different spore concentration of *B. bassiana*

Concentration, spores ml^{-1}	Cumulative percentage mortality at days after treatment		
	4	5	6
1.25×10^8	1.66	3.33	5.00
2.50×10^8	3.33	5.00	8.30
5.00×10^8	10.00	21.67	28.33
1.00×10^9	38.33	61.67	71.67
2.00×10^9	55.00	81.67	95.00
Control	1.66	1.66	1.66

Table 10. Probit analysis of dose mortality responses of third instar larvae of *S. litura* to varying doses of *B. bassiana*

Days after treatment	χ^2	Regression equation	LC_{50} spores/ml $\times 10^8$	Fiducial limit $\times 10^8$
4	2.1040	Y = 2.067 + 2.413 x	16.39	14.50 – 18.30
5	1.9810	Y = 2.171 + 2.948 x	9.10	8.40 – 9.82
6	2.7401	Y = 2.105 + 3.427 x	6.99	6.51 – 7.47

4.2.1.3 Pericallia ricini

B. bassiana caused mortality of the third instar larvae of *P. ricini* on the second day after inoculation. The extent of mortality caused by the fungus at concentrations varying from 1.25 x 10^5 to 2 x 10^6 spores ml^{-1} ranged from 23.33 to 73.33 per cent on the second day and 36.66 to 100 per cent on the third day after inoculation (Table 11). The LC_{50} value of the fungus reduced from 5.56 x 10^5 on the second day to 2.72 x 10^5 spores ml^{-1} on the third day. On the fourth day the percent mortality ranged from 50 to 100, where both the higher concentration (1 x 10^6 and 2 x 10^6 spores ml^{-1}) gave 100 per cent mortality. The fiducial limit calculated from the data indicated that for obtaining 50 per cent mortality on the second day, the spore concentration should be in the range of 4.44 x 10^5 to 6.69 x 10^5 spores ml^{-1}. Similarly the fiducial limit for the third day was between 2.43 x 10^5 to 3.01 x 10^5 spores ml^{-1} (Table 12).

4.2.2 *Fusarium pallidoroseum*

Spore concentrations ranging from 0.437 x 10^6 to 7 x 10^6 spores ml^{-1} of *F.pallidoroseum* was used for the bioassay of the fungus against the aphids *A. craccivora* and *M. persicae*.

4.2.2.1 Aphis craccivora

Cumulative per cent mortality of wingless adults of *A. craccivora* caused by *F. pallidoroseum* at different spore concentrations was used for probit analysis (Table 13). The mortality due to the fungus ranged from 5.00 to 86.67 per cent on the second day when the spray concentration increased from 0.437 x 10^6 to 7 x 10^6 spores ml^{-1}. The LC_{50} value calculated for the second day was 4.07 x 10^6 spores ml^{-1}. The fiducial limit calculated for the same day indicated that for obtaining 50 per cent mortality, the spore concentration should be within the range of 3.98 x 10^6 to 4.15 x 10^6 spores ml^{-1}. On the third day of inoculation, the mortality of aphids ranged from 6.67 to 92.50 per cent with increase in

Table 11. Cumulative percentage mortality of third instar larvae of *P. ricini* treated with different spore concentrations of *B. bassiana*

Concentration, spores ml^{-1}	Cumulative percentage mortality at days after treatment		
	2	3	4
1.25×10^5	23.33	36.66	50.00
2.50×10^5	30.00	76.67	50.00
5.00×10^5	46.66	80.00	98.00
1.00×10^6	66.67	96.66	100.00
2.00×10^6	73.33	100.00	100.00
Control	0	0	0

Table 12. Probit analysis of dose mortality responses of *P. ricini* to varying doses of *B. bassiana*

Days after treatment	χ^2	Regression equation	LC_{50} spores/ml $\times 10^5$	Fiducial limit $\times 10^5$
2	0.555	Y = 4.088 + 1.2230 x	5.56	4.44-6.69
3	2.675	Y = 4.213 + 2.414 x	2.72	2.43-3.01

the fungal spore concentration. The LC_{50} value was reduced to 3.86 x 10^6 spores ml^{-1} on the third day. Fiducial limits obtained indicated that the spore concentration ranging from 3.72 x 10^6 to 3.94 x 10^6 spores ml^{-1} was required for 50 per cent mortality on the third day of inoculation (Table 14).

4.2.2.2 Myzus persicae

Wingless adults of *M. persicae* were used for the bioassay. Death of aphids due to fungal infection was noticed from the second day onwards with mortality ranging from 30.83 to 98.33 per cent with spore concentrations ranging from 0.437 x 10^6 to 7 x 10^6 spores ml^{-1} (Table 15). The LC_{50} value for this period was 1.67 x 10^6 spores ml^{-1} with a fiducial limit between 1.55 x 10^6 to 1.78 x 10^6 spores ml^{-1}. The mortality per cent ranged from 39.16 to 100 per cent on the third day as the spore concentration increased from 0.437 x 10^6 to 7 x 10^6 spores ml^{-1}. The LC_{50} value on the third day was 0.72 x 10^6 with a fiducial limit of 0.66 x 10^6 to 0.77 x 10^6 spores ml^{-1} (Table 16).

4.2.3 *Rhizopus oryzae*

Bioassay of *R. oryzae* was carried out using third instar nymphs of *D. cingulatus*. The fungus caused death of the bugs on the 5th day onwards. The per cent mortality ranged from 0 to 73.33 on the 5th day, 8.33 to 86.66 per cent on the 6th day and 11.66 to 93.33 on the 7th day of inoculation, with spore concentrations ranging from 1.25 x 10^6 to 2 x 10^7 spores ml^{-1} (Table 17). The uninfected nymphs entered into 4th instar and completed its life cycle normally. The LC_{50} values showed gradual reduction from 7.42 x 10^6 to 4.15 x 10^6 spores ml^{-1} on 5th day to 7th day after inoculation. Fiducial limits calculated for 50 per cent mortality on the 7th day was between 3.76 x 10^6 to 4.54 x 10^6 spores ml^{-1} (Table 18).

Table 13. Cumulative per cent mortality of *A. craccivora* treated with different spore concentrations of *F. pallidoroseum*

Concentration, spores ml^{-1}	Cumulative percentage mortality at days after treatment		
	1	2	3
0.437×10^6	2.50	5.00	6.67
0.875×10^6	2.50	10.00	14.17
1.75×10^6	5.00	30.83	38.33
3.50×10^6	10.82	39.17	73.33
7.00×10^6	12.50	86.67	92.50
Control	0	2.50	2.50

Table 14. Probit analysis of dose mortality responses of *A. craccivora* to varying doses of *F. pallidoroseum*

Days after treatment	χ^2	Regression equation	LC_{50} spores/ml $\times 10^6$	Fiducial limit $\times 10^6$
2	21.6530	Y=1.0098+6.543x	4.07	3.98-4.15
3	14.2239	Y=1.2180+6.441x	3.86	3.72-3.94

Table 15. Cumulative per cent mortality of *M. persicae* treated with different spore concentrations of *F. pallidoroseum*

Concentration, spores ml^{-1}	Cumulative per cent mortality at days after treatment	
	2	3
0.437×10^6	30.83	39.16
0.875×10^6	38.33	73.33
1.75×10^6	45.83	80.00
3.50×10^6	85.83	98.33
7.00×10^6	98.33	100.00
Control	15.83	17.50

Table 16. Probit analysis of dose mortality responses of *M. persicae* to varying doses of *F. pallidoroseum*

Days after treatment	χ^2	Regression equation	LC_{50} spores/ml $\times 10^6$	Fiducial limit $\times 10^6$
2	19.453	Y =4.4180 + 2.6236 x	1.67	1.55-1.78
3	9.517	Y =5.3740 + 2.5960 x	0.72	0.66-0.77

Table 17. Cumulative per cent mortality of *D. cingulatus* treated with different spore concentrations of *R. oryzae*

Concentration, spores ml^{-1}	Cumulative percentage mortality at days after treatment		
	5	6	7
1.25×10^6	0	8.33	11.66
2.50×10^6	25.00	31.66	41.66
5.00×10^6	41.66	46.66	55.00
1.00×10^7	63.33	56.66	80.00
2.00×10^7	73.33	86.66	93.33
Control	0	3.33	3.33

Table 18. Probit analysis of dose mortality responses of *D. cingulatus* to varying doses of *R. oryzae*

Days after treatment	χ^2	Regression equation	LC_{50} spores/ml $\times 10^6$	Fiducial limit $\times 10^6$
5	8.5366	Y=3.327+1.921x	7.42	6.66-8.17
6	5.1327	Y=3.496+1.874x	6.34	6.55-6.85
7	2.6649	Y=3.656+2.172x	4.15	3.76-4.54

4.3 MASS MULTIPLICATION OF ENTOMOPATHOGENIC FUNGI

Mycelial growth and sporulation of entomopathogenic fungi *viz.*, *B. bassiana*, *F. pallidoroseum* and *R. oryzae* grown in rice bran, gingelly oil cake, coconut water, starch solution and plain water was recorded.

4.3.1 *Beauveria bassiana*

B. bassiana exhibited profuse growth on ricebran, gingelly oil cake and coconut water and its growth in starch solution was moderate (Plate 7). A marked difference in the spore count of the fungus in different substrates was noticed with rice bran giving the maximum (19.25 x 10^7 spores ml^{-1}) and starch solution the least (3.24 x 10^7 spores ml^{-1}) (Table 19 and Fig. 1). The fungus failed to grow in water.

4.3.2 *Fusarium pallidoroseum*

The mycelial growth of *F. pallidoroseum* was profuse on ricebran, gingelly oil cake, coconut water and moderate in starch solution (Plate 8). Production of fungal spores in ricebran (9.82 x 10^7 spores ml^{-1}) and gingelly oil cake (8.98 x 10^7 spores ml^{-1}) were on par to each other. However there was no significant difference between the spore count observed in coconut water (6.39 x 10^7 spores ml^{-1}) and starch solution (6.18 x 10^7 spores ml^{-1}) eventhough coconut water supported better mycelial growth (Table 20 and Fig. 2).

4.3.3 *Rhizopus oryzae*

Profuse growth of *R. oryzae* was observed in rice bran and gingelly oil cake while it exhibited only moderate growth in coconut water and starch solution (Plate 9). Sporulation of the fungus varied significantly among the treatments with rice bran giving maximum spore load (18.90 x 10^7 spores ml^{-1}) followed by gingelly oil cake (15.36 x 10^7 spores ml^{-1}). Starch solution recorded the least number of spores (9.10 x 10^7 spores ml^{-1}). Fungus did not grow in water (Table 21 and Fig. 3).

Table 19. Mycelial growth and spore count of *B. bassiana* in different substrates

Sl. No.	Substrate	Mycelial growth rate*	Spore count / ml x 10^7
1	Rice bran	+++	19.25 (4.50)
2	Gingelly oil cake	+++	11.67 (3.56)
3	Coconut water	+++	4.56 (2.36)
4	Starch solution	++	3.24 (2.06)
5	Water	0	0 (1)

CD for comparison of spore count – (0.08)

Table 20. Mycelial growth and spore count of *F. pallidoroseum* in different substrates

Sl. No.	Substrate	Mycelial growth rate*	Spore count / ml x 10^7
1	Rice bran	+++	9.82 (3.29)
2	Gingelly oil cake	+++	8.98 (3.16)
3	Coconut water	+++	6.39 (2.72)
4	Starch solution	++	6.18 (2.68)
5	Water	0	0 (1)

CD for comparison of spore count – (0.22)

Figures in parentheses are values after $\sqrt{x+1}$ transformation

* +++ - Profuse growth

++ - Moderate growth

\+ - Slight growth

0 - no growth

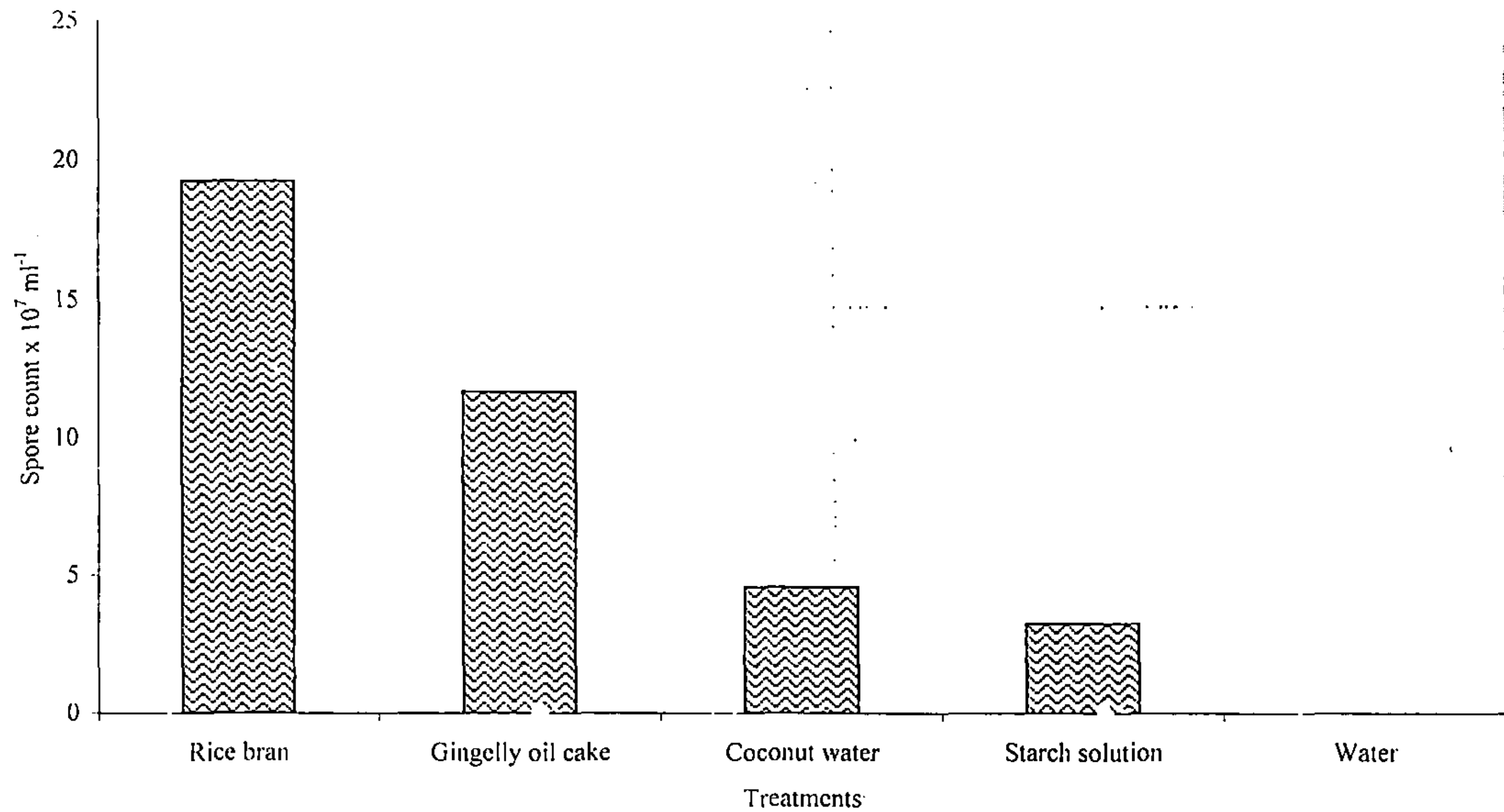

Fig. 1. Spore count (eleven days after inoculation) of *B.bassiana* in different substrates

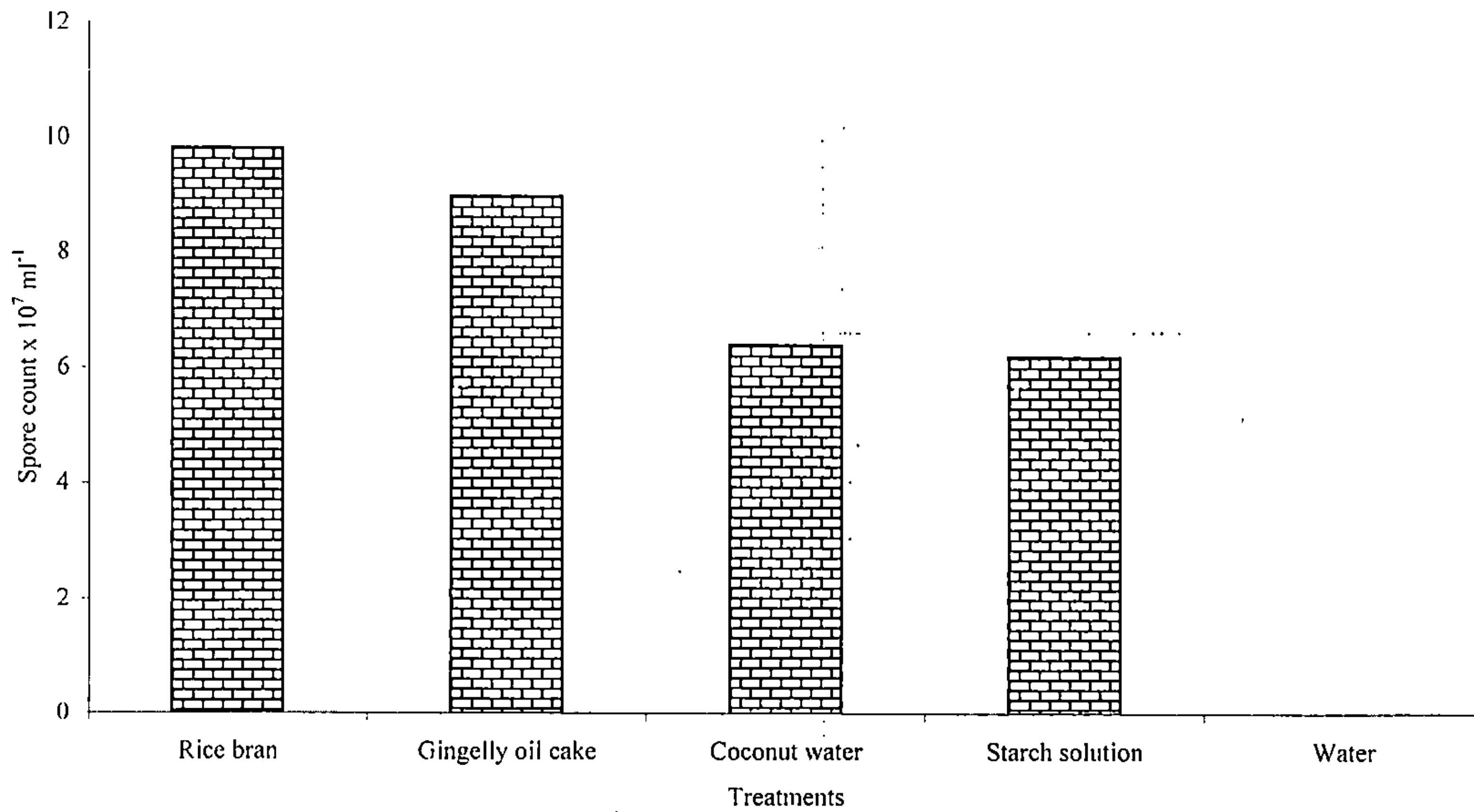

Fig. 2. Spore count (seven days after inoculation) of *F. pallidoroseum* in different substrates

Table 21. Mycelial growth and spore count of *R. oryzae* in different substrates

Sl. No.	Substrate	Mycelial growth rate*	Spore count / ml x 10^7
1	Rice bran	+++	18.90 (4.46)
2	Gingelly oil cake	+++	15.36 (4.04)
3	Coconut water	++	11.07 (3.48)
4	Starch solution	++	9.10 (3.18)
5	Water	0	0 (1)

CD for comparison of spore count – (0.02)

Figures in parentheses are values after $\sqrt{x+1}$ transformation

* +++ - Profuse growth

++ - Moderate growth

\+ - Slight growth

0 - no growth

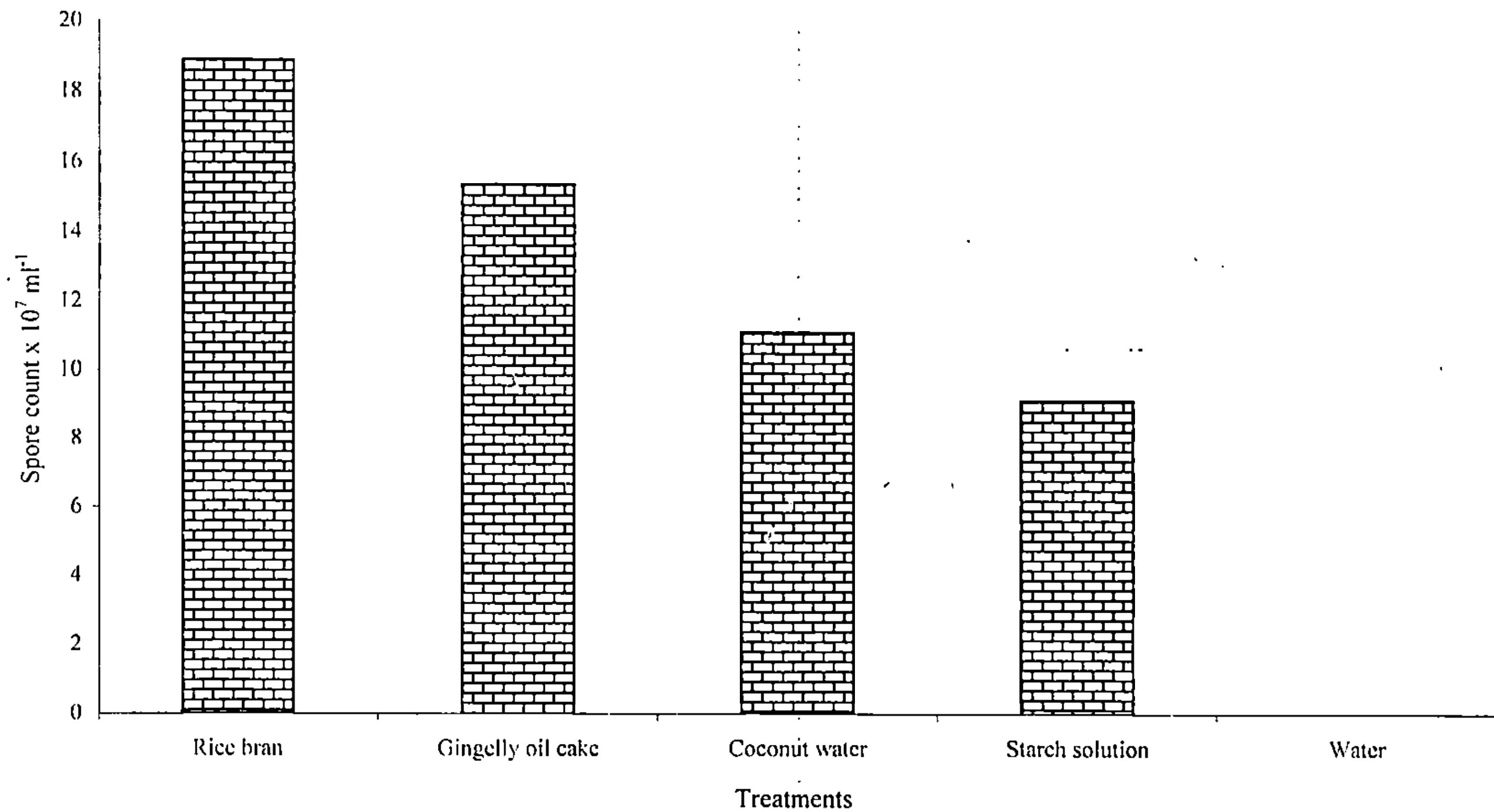

Fig. 3. Spore count (six days after inoculation) of *R. oryzae* in different substrates

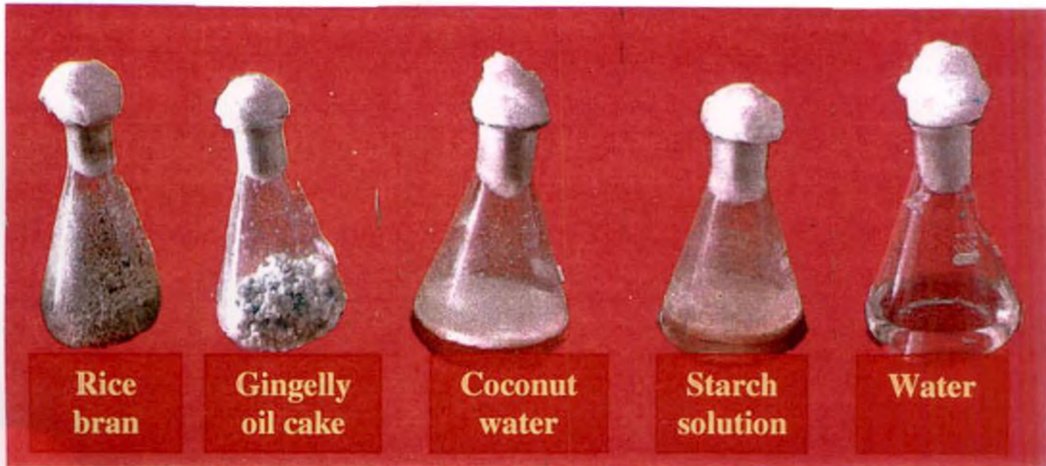

Plate 7. Growth of *B.bassiana* in different solid and liquid substrates

Plate. 8. Growth of *F.pallidoroseum* on different solid and liquid substrates

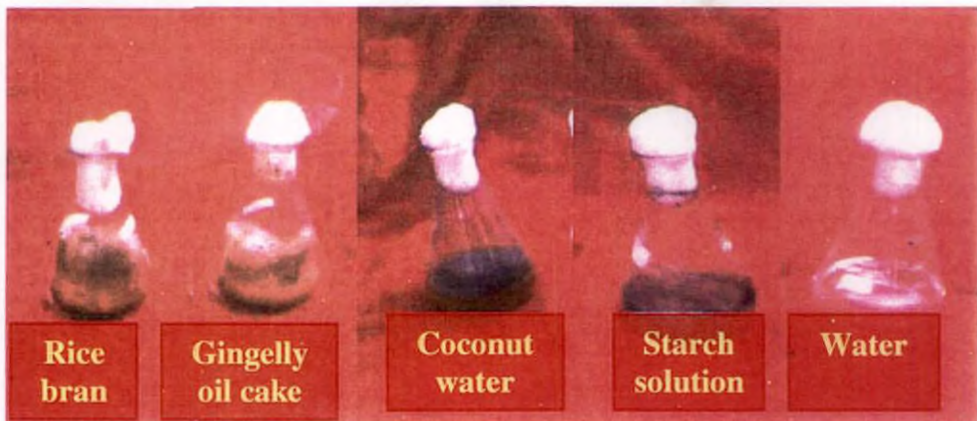

Plate. 9 Growth of *R.oryzae* in different solid and liquid substrates

4.4 SHELF LIFE STUDIES

Effect of period of storage at room temperature on the viability and virulence of *B. bassiana, F. pallidoroseum* and *R. oryzae* stored in rice bran, gingelly oil cake, coconut water and starch solution was studied at monthly intervals.

4.4.1 *Beauveria bassiana*

4.4.1.1 Effect on viability

The fresh culture (control) of *B. bassiana* could grow to 85.75 mm in a 9 cm petridish within ten day period (Table 22). When the fungus was stored for different periods of time in different media, a reduction in the growth rate was observed. The fungus stored in starch solution, failed to grow on PDA after two months but when stored in coconut water, it retained its viability till the end of the third month (6.25 mm). Rice bran and gingelly oil cake, eventhough supported growth upto the end of five months, there was marked reduction in their growth rate. The growth rate observed from these two growing media did not differ significantly from the control at the end of first month. However from the second month onwards, these two media supported a lesser growth than control with 11.59 to 66.92 per cent inhibition of growth over control. But there was no significant difference in the growth rate when *B. bassiana* was stored in these two media. For coconut water (70.75 mm) and starch solution (70.50 mm) even at the end of first month of storage, the growth rate was significantly less than that observed in control (Fig. 4).

4.4.1.2 Effect on spore count

The spore count of the fungus grown in different media was estimated for a period of six months. Gradual reduction in the spore count of the fungus, in various storage media, was noticed when it was stored for extended period.

Table 22. Effect of period of storage (at room temperature) on the viability of *B. bassiana*

MAS	Rice bran		Gingelly oil cake		Coconut water		Starch solution		Control
	MCD (mm)	PIOC	MCD (mm)	PIOC	MCD (mm)	PIOC	MCD (mm)	PIOC	MCD (mm)
1	83.15	3.34 (1.91)	82.75	3.78 (2.12)	70.75	17.73 (10.19)	70.50	18.02 (10.38)	86.00
2	68.90	20.13 (11.59)	65.00	24.85 (1.43)	33.00	61.85 (37.59)	28.05	67.63 (42.55)	86.50
3	60.50	30.05 (17.49)	56.80	34.77 (20.30)	6.25	92.38 (63.37)	0	100.00 (90.00)	87.00
4	51.00	40.52 (23.89)	50.15	41.10 (24.26)	0	100.00 (90.00)	0	100.00 (90.00)	85.75
5	7.00	91.86 (66.63)	6.80	92.00 (66.92)	0	100.00 (90.00)	0	100.00 (90.00)	86.00
6	0	100.00 (90.00)	0	100.00 (90.00)	0	100.00 (90.00)	0	100.00 (90.00)	86.00

MAS – Months after storage
MCD – Mean colony diameter
PIOC – Percentage inhibition over control
Values in parentheses are after angular transformation

CD for comparison of treatment combinations – (8.910)

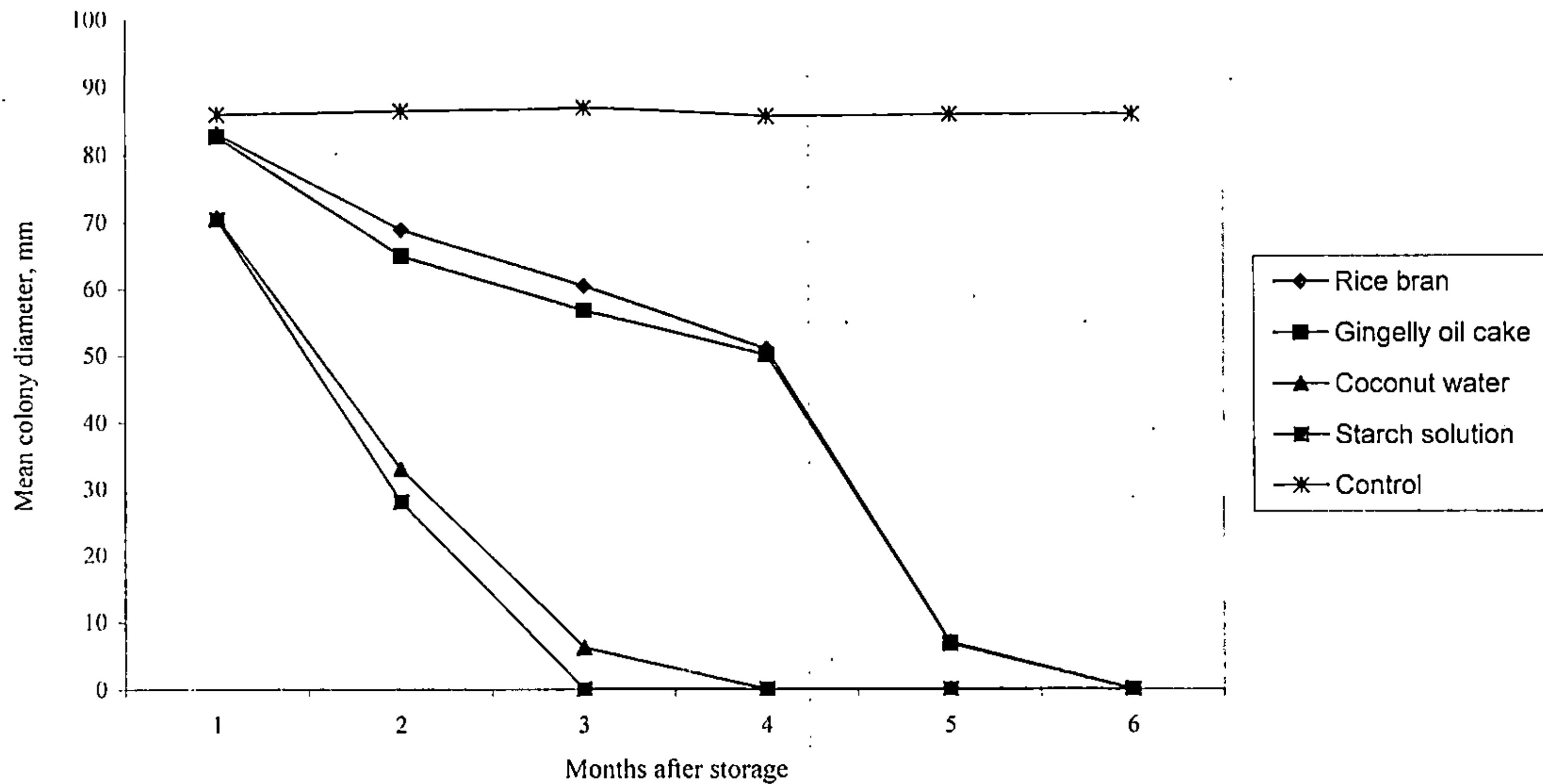

Fig. 4. Effect of period of storage on viability of *B.bassiana* (at room temperature)

B. bassiana stored in coconut water failed to sporulate from 4th month onwards while, when the fungus was stored in starch solution, sporulation was absent even from third month. Both rice bran and gingelly oil cake supported the sporulation of the fungus upto a period of five months. There was no significant difference in the extent of sporulation among these two storage media over a period of five months (Table 23).

Spore count observed in coconut water and starch solution also did not differ significantly during different periods of storage. These two media during the entire period of time supported less spore count compared to rice bran and gingelly oil cake.

4.4.1.3 Effect on virulence

To find out the virulence of the fungus stored in different media at varying period of time, its pathogenicity to 4th instar larvae of *S. derogata* was estimated. The fungus grown on ricebran and gingelly oil cake could effect more than 90 per cent mortality of the insect at the end of one month of storage (Table 24). During this period, the mortality rate due to the fungus grown in coconut water and starch solution were 42.75 and 24.05 respectively and after third month onwards they failed to cause death of the test insect. The mortality rate caused by the fungus grown on rice bran and gingelly oil cake upto the end of three months was more than 75 per cent. However a drastic reduction was noticed from the end of 4th month onwards and it failed to cause death of the insect after five months of storage. The rate of pathogenicity of the fungus grown on rice bran and gingelly oil cake did not differ significantly during the different periods of storage (Fig. 5).

Table 23. Effect of period of storage (at room temperature) on sporulation of *B. bassiana* (spores ml^{-1}) x 10^6

MAS	Rice bran	Gingelly oil cake	Coconut water	Starch solution
1	22.75 (4.87)	21.02 (4.69)	5.10 (2.47)	1.61 (1.61)
2	21.53 (4.75)	20.44 (4.63)	1.42 (1.55)	1.04 (1.43)
3	20.04 (4.59)	19.60 (4.54)	1.40 (1.55)	0 (1.00)
4	15.21 (4.03)	14.51 (3.94)	0 (1.00)	0 (1.00)
5	12.67 (3.69)	9.48 (3.24)	0 (1.00)	0 (1.00)
6	0 (1.00)	0 (1.00)	0 (1.00)	0 (1.00)

CD for comparison of treatment combinations – (0.85)

Values in parentheses are after $\sqrt{x+1}$ transformation

MAS – Months after storage

Table 24. Effect of period of storage (at room temperature) on the virulence of *B. bassiana*

MAS	Percentage mortality of fourth instar larvae of *S. derogata*				
	Rice bran	Gingelly oil cake	Coconut water	Starch solution	Control
1	97.00 (75.93)	93.50(69.23)	42.75(25.28)	24.05(13.92)	1.00(0.57)
2	85.00(58.21)	86.75(60.11)	26.06(14.48)	8.56 (04.91)	0 (0.04)
3	80.75(53.13)	75.02(48.59)	0(0.04)	0 (0.04)	0 (0.04)
4	42.50(25.15)	38.58(22.64)	0(0.04)	0 (0.04)	0 (0.04)
5	10.45(05.99)	9.98(05.73)	0(0.04)	0 (0.04)	0 (0.04)
6	0(0.04)	0(0.04)	0(0.04)	0 (0.04)	0 (0.04)

CD for comparison of treatment combinations – (7.015)

MAS–months after storage

Values in parentheses are after angular transformation

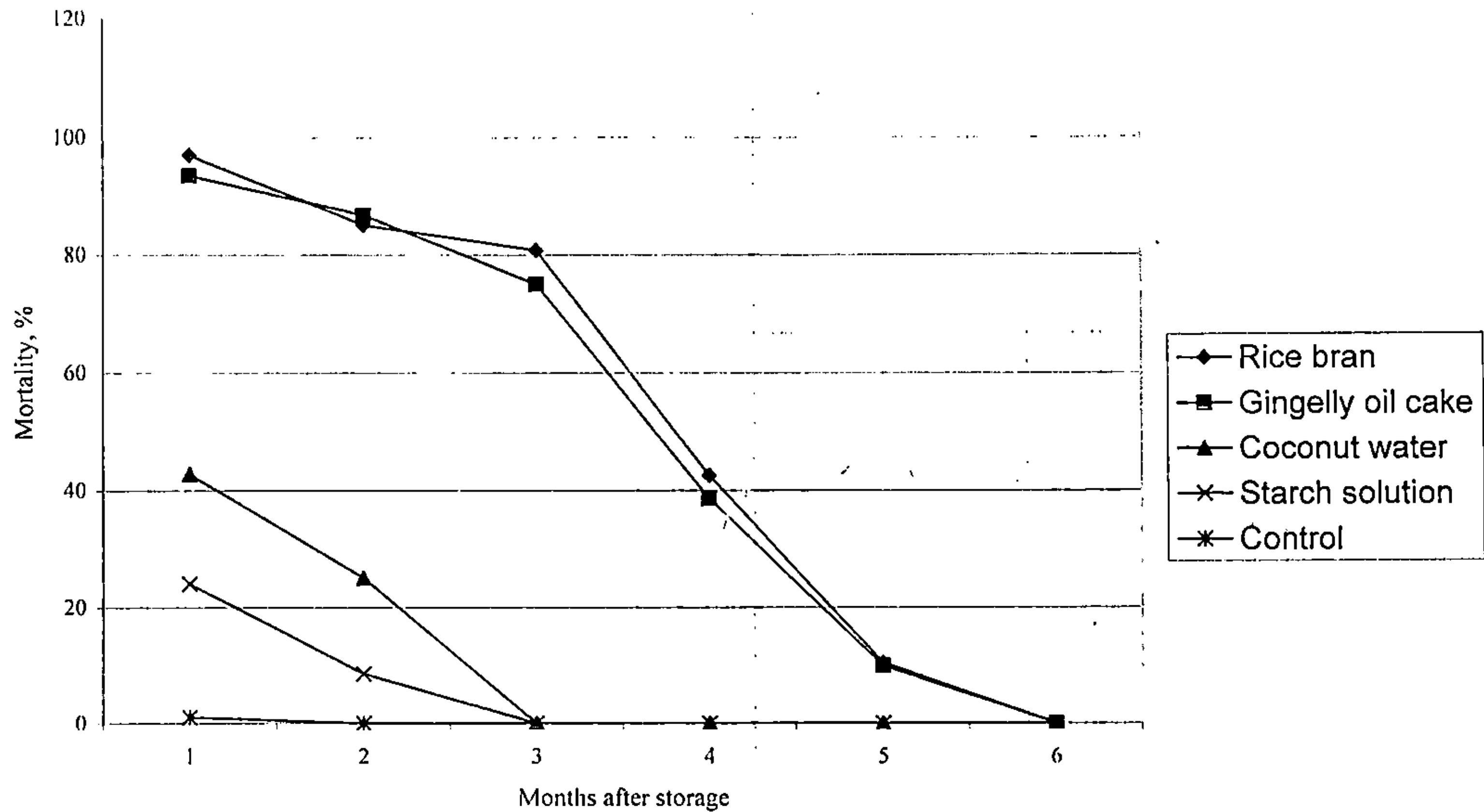

Fig. 5. Effect of period of storage on virulence of *B.bassiana* (at room temperature)

4.4.2 *Fusarium pallidoroseum*

4.4.2.1 Effect on viability

The mean colony diameter of the fungus stored in different media was measured at the end of seven days after inoculation and per cent inhibition over control was calculated.

The extent of growth of *F. pallidoroseum* at the end of one month after storage was significantly less than that observed in control. From the second month onwards the colony diameter of the fungus grown in liquid cultures (coconut water and starch solution) was significantly less than that observed when the fungus was grown on solid substrates (rice bran and gingelly oil cake) (Table 25).

The extent of reduction ranged from 18.89 in starch solution to 11.11 per cent in rice bran at the end of first month. Second month onwards, the reduction in the colony diameter ranged from 57.08 in starch solution to 16.67 in rice bran. However there was no significant difference in the colony diameter between the treatments rice bran and gingelly oil cake and between coconut water and starch solution (Fig. 6).

From the 3rd month onwards fungus failed to grow when it was stored in liquid media. Both the solid media supported growth even upto the end of six month, even though, there was a marked reduction in the colony diameter as the storage time increased. At the end of six months, the extent of reduction in the growth of fungus grown in gingelly oil cake and rice bran were 84.44 and 81.25 per cent over the control respectively. There was no significant difference between the colony diameter observed in rice bran and gingelly oil cake during the entire period of storage.

4.4.2.2 Effect on spore count

The spore count of the fungus in different storage media showed gradual reduction as the length of storage increased (Table 26). At the end of first month of storage, there was no significant difference between

Table 25. Effect of period of storage (at room temperature) on the viability of *F. pallidoroseum*

MAS	Rice bran		Gingelly oil cake		Coconut water		Starch solution		Control
	MCD (mm)	PIOC	MCD (mm)	PIOC	MCD (mm)	PIOC	MCD (mm)	PIOC	MCD (mm)
1	80.00	11.11 (06.38)	79.25	11.94 (06.86)	76.50	14.99 (08.62)	73.00	18.89 (10.89)	90.00
2	75.00	16.67 (09.59)	73.50	18.33 (10.56)	43.00	52.22 (34.48)	39.50	57.08 (34.81)	90.00
3	70.75	21.39 (12.35)	65.75	26.95 (15.63)	0	100 (90.00)	0	100 (90.00)	90.00
4	50.85	47.22 (28.18)	42.00	53.33 (32.23)	0	100 (90.00)	0	100 (90.00)	90.00
5	20.00	77.77 (51.05)	18.00	79.99 (53.12)	0	100 (90.00)	0	100 (90.00)	90.00
6	16.87	81.25 (54.34)	13.00	84.44 (57.61)	0	100 (90.00)	0	100 (90.00)	90.00

MAS – Months after storage
MCD – Mean colony diameter
PIOC – Percentage inhibition over control
Values in parentheses are after angular transformation

CD for comparison of treatment combinations – (5.325)

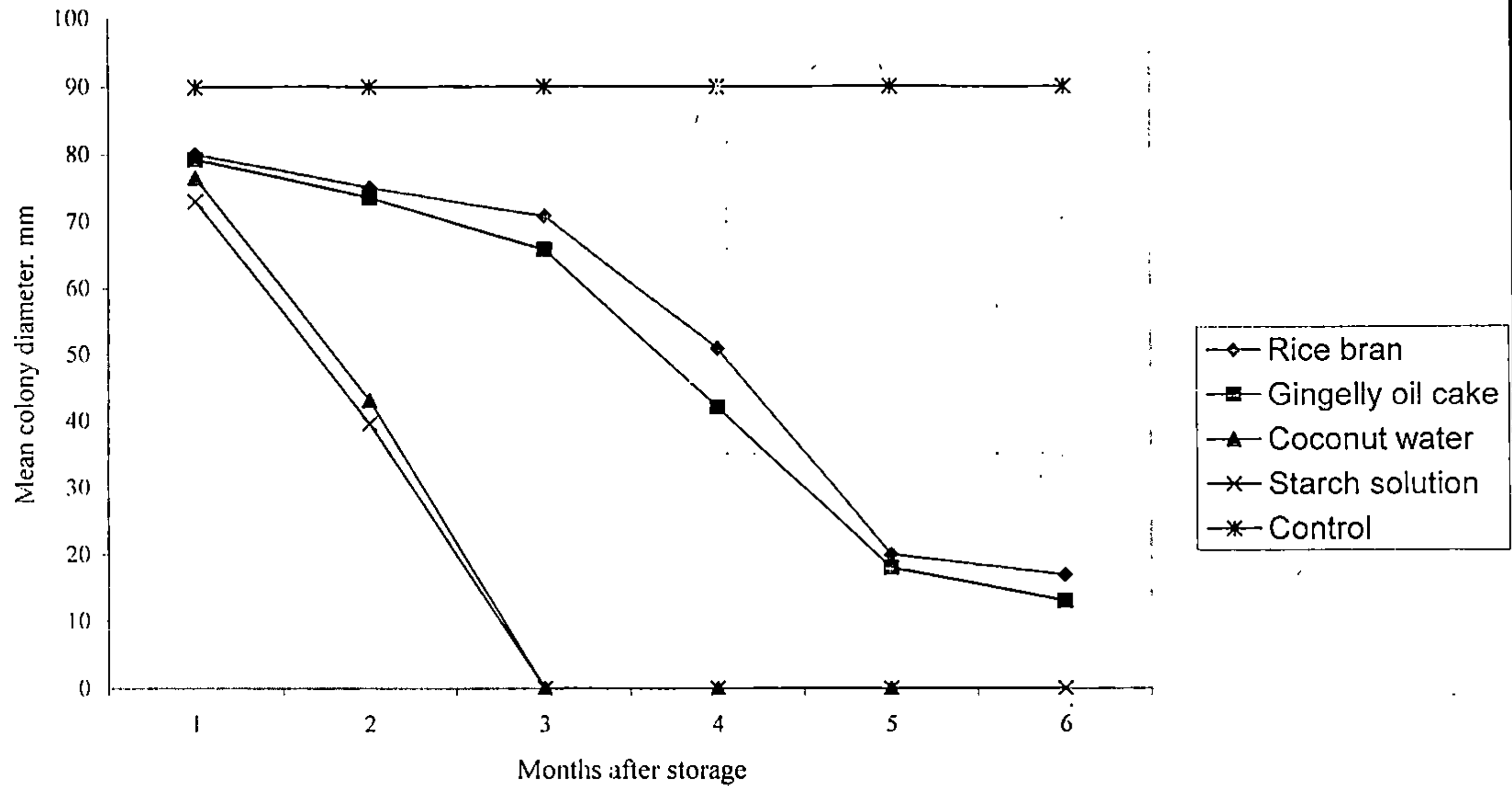

Fig. 6. Effect of period of storage on viability of *F.pallidoroseum* (at room temperature)

Table 26. Effect of period of storage (at room temperature) on sporulation *of F. pallidoroseum* (spores ml^{-1}) x 10^6

MAS	Rice bran	Gingelly oil cake	Coconut water	Starch solution
1	2.51 (1.87)	2.57 (1.89)	1.91 (1.48)	1.07 (1.44)
2	2.03 (1.74)	2.08 (1.75)	0.95 (1.39)	0.52 (1.23)
3	2.01 (1.73)	1.96 (1.72)	0 (1.00)	0 (1.00)
4	1.84 (1.68)	1.56 (1.60)	0 (1.00)	0 (1.00)
5	1.56 (1.60)	0.96 (1.40)	0 (1.00)	0 (1.00)
6	1.20 (1.48)	0.84 (1.36)	0 (1.00)	0 (1.00)

CD for comparison of treatment combinations – (0.915)

MAS–months after storage

Values in the parentheses are after $\sqrt{x + 1}$ transformation

the treatments and the spore count varied from 1.07 x 10^6 spores ml^{-1} (starch solution) to 2.57 x 10^6 spores ml^{-1} (gingelly oil cake).

Only rice bran and gingelly oil cake supported growth of fungus beyond two months of storage. There was no significant difference between these two media. The number of spores at the end of six month in rice bran was 1.20 x 10^6 spores ml^{-1} and that in gingelly oil cake was 0.84 x 10^6 spores ml^{-1} respectively.

4.4.2.3 Effect on virulence

Virulence of *F. pallidoroseum* grown in different media was estimated periodically by testing their pathogenicity on wingless adults of *A. craccivora*. At the end of first month of storage, rice bran (75.00 %) and gingelly oil cake (65.05 %) gave maximum per cent mortality while the virulence of fungi grown in coconut water (48.08 %) and starch solution (45.50 %) reduced considerably (Table 27 and Fig. 7).

There was further reduction in the virulence of the fungus in all the media, at the end of second month of storage, with per cent mortality ranging from 9.75 (starch solution) to 65.35 (rice bran). Only the fungus grown in solid media (rice bran and gingelly oil cake) retained the virulence beyond two months of storage.

Virulence of the fungus grown in rice bran and gingelly oil cake reduced considerably at the end of six months (25.75 and 24.85 per cent mortality respectively) but there was no significant difference in the virulence between these two storage media.

4.4.3 *Rhizopus oryzae*

4.4.3.1 Effect on viability

R. oryzae could cover 9 cm petriplate completely on the third day of inoculation in control. At the end of first month of storage, fungus grown in different media did not differ significantly from the control. During the second month also a similar trend was noticed. However,

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Table 27. Effect of period of storage (at room temperature) on virulence of *F. pallidoroseum*

MAS	Percentage mortality of *A. craccivora*				
	Rice bran	Gingelly oil cake	Coconut water	Starch solution	Control
1	75.00(48.59)	65.05(40.58)	48.05(28.72)	45.50(27.06)	1.05(0.60)
2	65.35(40.81)	59.58(36.57)	12.50(7.18)	9.75(5.59)	2.75(1.57)
3	58.87(35.85)	55.75(33.88)	1.00(0.57)	1.62(0.92)	0(0.04)
4	46.50(27.71)	43.05(25.49)	1.00(0.57)	0.92(0.53)	0.98(0.56)
5	40.25(23.73)	38.75(22.79)	0.80(0.46)	1.02(0.58)	0.75(0.43)
6.	25.75(14.92)	24.85(14.39)	1.25(0.72)	1.14(0.65)	1.14(0.65)

CD for comparison of treatment combinations – (12.850)

MAS–months after storage

Values in parentheses are after angular transformation

Mortality, %

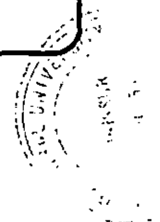
80
70
60
50
40
30
20
10
0

1 2 3 4 5 6

Months after storage

Rice bran
Gingelly oil cake
Coconut water
Starch solution
Control

Fig. 7. Effect of period of storage on virulence of *F.pallidoroseum* (at room temperature)



starch solution with 12.78 per cent reduction in the growth over the control was inferior to the other treatments (Table 28 and Fig. 8).

The viability of the fungus grown in coconut water and starch solution reduced significantly at the end of third month of storage and was lost completely at the end of fourth month. There was significant reduction in the colony diameter of the fungus grown in rice bran and gingelly oil cake over the period of time. Rice bran gave 42.22 per cent inhibition of fungal growth over control and gingelly oil cake 43.33 per cent at the end of sixth month of storage.

4.4.3.2 Effect on spore count

Spore count of *R. oryzae* stored in different media showed a gradual reduction over the period of storage (Table 29). However, the reduction of spore count was not significant in rice bran and gingelly oil cake over the entire period of storage. The spore count observed in coconut water at the end of three months (2.40×10^7 spores ml^{-1}) was significantly lesser than that recorded at the end of first month of storage (6.44×10^7 spores ml^{-1}). Similarly, the spore count of *R. oryzae* in starch solution reduced significantly from 5.02×10^7 to 1.06×10^7 spores ml^{-1}.

4.4.3.3 Effect on virulence

Virulence of the fungus *R. oryzae* grown and stored in different media was estimated periodically by testing its pathogenicity to the third instar nymphs of *D. cingulatus*. None of the media retained the virulence of the fungus beyond three months of storage (Table 30 and Fig. 9).

Per cent mortality produced by fungus stored in rice bran reduced significantly at the end of three months (25 %) compared to that at the end of first month (60 %). Similar trend was observed in the case of gingelly oil cake also with per cent mortality reducing from 58.25 to 19.75.

Table 28. Effect of period of storage (at room temperature) on the viability of *R. oryzae*

MAS	Rice bran		Gingelly oil cake		Coconut water		Starch solution		Control
	MCD (mm)	PIOC	MCD (mm)	PIOC	MCD (mm)	PIOC	MCD (mm)	PIOC	MCD (mm)
1	90.00	0	90.00	0	90.00	0	87.00	3.33 (1.90)	90.00
2	85.00	5.55 (3.18)	87.00	3.33 (1.90)	80.00	11.11 (6.38)	78.50	12.78 (7.34)	90.00
3	82.00	8.89 (5.10)	82.00	8.89 (5.10)	70.00	22.22 (12.84)	65.00	27.78 (16.13)	90.00
4	80.00	11.11 (6.38)	79.00	12.22 (7.02)	0	100 (90.00)	0	100 (90.00)	90.00
5	78.00	13.33 (7.66)	75.00	16.67 (9.59)	0	100 (90.00)	0	100 (90.00)	90.00
6	52.00	42.22 (24.97)	51.00	43.33 (25.68)	0	100 (90.00)	0	100 (90.00)	90.00

MAS – Months after storage
MCD – Mean colony diameter
PIOC – Percentage inhibition over control
Values in parentheses are after angular transformation

CD for comparison of treatment combinations – (10.987)

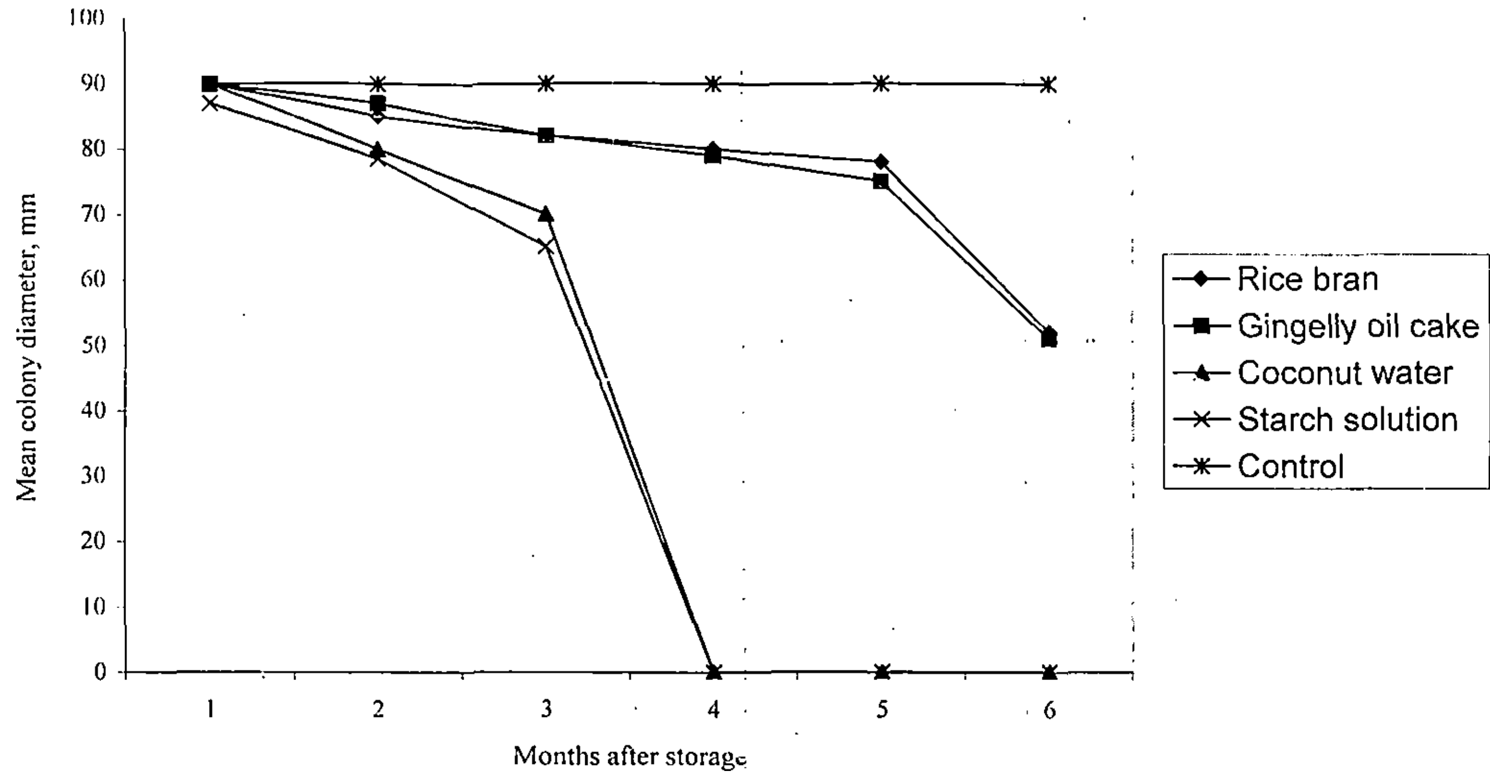


Fig. 8. Effect of period of storage on viability of *R.oryzae* (at room temperature)

Table 29. Effect of period of storage (at room temperature) on sporulation of *R. oryzae* (spores ml^{-1}) x 10^7

MAS	Rice bran	Gingelly oil cake	Coconut water	Starch solution
1	10.50 (3.39)	10.80 (3.43)	6.44 (2.73)	5.02 (2.45)
2	9.57 (3.25)	10.02 (3.32)	5.56 (2.56)	4.46 (2.34)
3	9.01 (3.16)	8.96 (3.15)	2.40 (1.84)	1.06 (1.43)
4	7.01 (2.83)	7.58 (2.93)	0 (1.00)	0 (1.00)
5	6.56 (2.75)	6.54 (2.74)	0 (1.00)	0 (1.00)
6	6.54 (2.74)	6.02 (2.65)	0 (1.00)	0 (1.00)

CD for comparison of treatment combinations – (0.826)

MAS–months after storage

Values in parentheses are after $\sqrt{x + 1}$ transformation

Table 30. Effect of period of storage (at room temperature) on virulence of *R. oryzae*

MAS	Percentage mortality of *D. cingulatus*				
	Rice bran	Gingelly oil cake	Coconut water	Starch solution	Control
1	60.00(36.87)	58.25(35.63)	40.00(23.58)	38.50(22.64)	0 (0.04)
2	51.00(30.66)	56.00(34.05)	20.00(11.54)	10.00(05.74)	0 (0.04)
3	25.00(14.48)	19.75(11.39)	0 (0.04)	0 (0.04)	0(0.04)
4	0 (0.04)	0 (0.04)	0 (0.04)	0 (0.04)	0 (0.04)
5	0 (0.04)	0 (0.04)	0 (0.04)	0 (0.04)	0 (0.04)
6	0 (0.04)	0 (0.04)	0 (0.04)	0 (0.04)	0 (0.04)

CD for comparison of treatment combinations – (12.850)

MAS–months after storage

Values in parentheses are after angular transformation

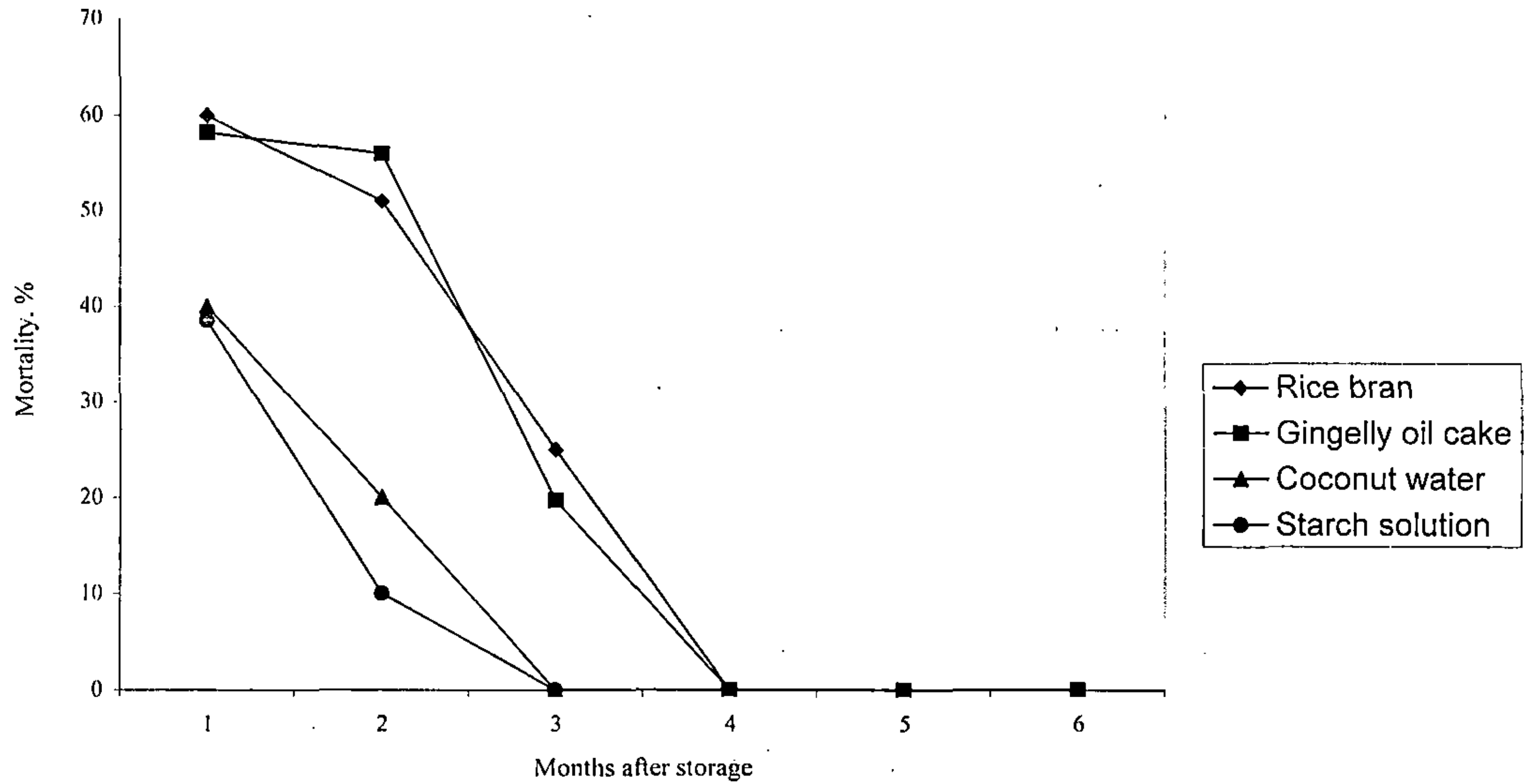


Fig. 9. Effect of period of storage on virulence of *R.oryzae* (at room temperature)

Coconut water and starch solution could produce 40 and 38.50 per cent mortality at the end of first month of storage, and 20 and 10 per cent at the end of second month of storage. These liquid media could not retain the virulence of the fungus at the end of third month of storage.

4.5 COMPATIBILITY OF ENTOMOPATHOGENIC FUNGI WITH PESTICIDES

The effect of different pesticides on growth and sporulation of the fungi *B. bassiana, F. pallidoroseum* and *R. oryzae* was studied on PDA using Poison Food Technique.

4.5.1 *Beauveria bassiana*

B. bassiana completely covered a 9 cm petridish within 11 days after inoculation in the control, where the fungus was grown on PDA (Plate 10a). The growth of the fungus in the media poisoned with two per cent neem oil emulsion behaved similar to the control. Dimethoate at 0.05 per cent completely inhibited the fungal growth. In all the other treatments the growth of the fungus was restricted with per cent inhibition ranging from 79.25 to 96.67 (Table 31 and Fig. 10). All the pesticides tried, considerably reduced the sporulation of the fungus. Among the pesticides which supported the fungal growth, the per cent decrease in sporulation was least in the case of two per cent neem oil emulsion (3.83) and highest in the case of quinalphos (98.71).

4.5.2 *Fusarium pallidoroseum*

F. pallidoroseum covered a 9 cm petridish within nine days when it was grown on PDA without incorporating any pesticides (Plate 10b). The growth of the fungus was completely inhibited in the case of media poisoned with dimethoate 0.05 per cent (Fig.11). Among the other pesticides, the maximum inhibition of mycelial growth was noticed in media incorporated with two per cent neem oil emulsion (66.29) followed by quinalphos (61.84) (Table 32). There was no significant

Table 31. Effect of different pesticides on mycelial growth and sporulation of *B. bassiana*

Sl.No.	Pesticides	% inhibition of mycelial growth	% decrease of spore count
1.	Chlorpyriphos 0.05 %	79.25 (62.63)	95.91 (78.46)
2.	Malathion 0.1 %	81.84 (64.82)	97.95 (81.85)
3.	Quinalphos 0.05 %	88.51 (70.19)	98.71 (83.54)
4.	Dimethoate 0.05 %	100.00 (90.00)	100.00 (90.00)
5.	Mancozeb 0.2 %	96.67 (75.17)	97.75 (77.78)
6.	Neem oil emulsion 2 %	0 (0.035)	3.83 (11.09)
	CD	(3.23)	(3.58)

Figures in parentheses are values after angular transformation

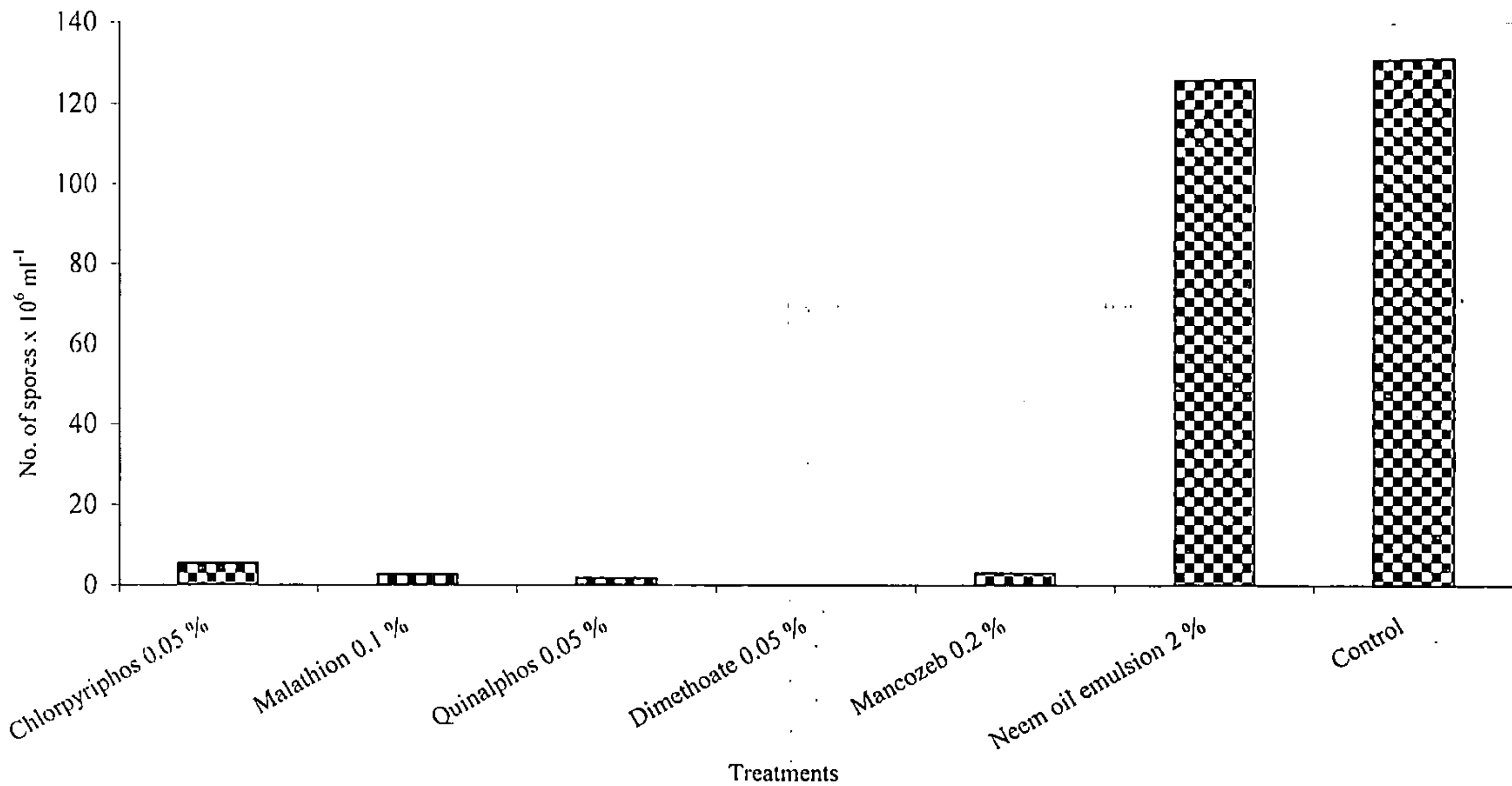


Fig. 10 Spore count (eleven days after inoculation) of *B.bassiana* on PDA poisoned with different pesticides

Table 32. Effect of different pesticides on mycelial growth and sporulation of *F. pallidoroseum*

Sl.No.	Pesticides	% inhibition of mycelial growth	% decrease of spore count
1.	Chlorpyriphos 0.05 %	51.10 (45.61)	45.45 (42.34)
2.	Malathion 0.1 %	52.35 (46.25)	69.69 (56.62)
3.	Quinalphos 0.05 %	61.84 (51.84)	69.69 (56.62)
4.	Dimethoate 0.05 %	100.00 (90.00)	100.00 (90.00)
5.	Mancozeb 0.2 %	55.55 (48.17)	75.75 (60.57)
6.	Neem oil emulsion 2 %	66.29 (54.49)	78.78 (62.65)
	CD	(1.805)	(6.269)

Figures in parentheses are values after angular transformations

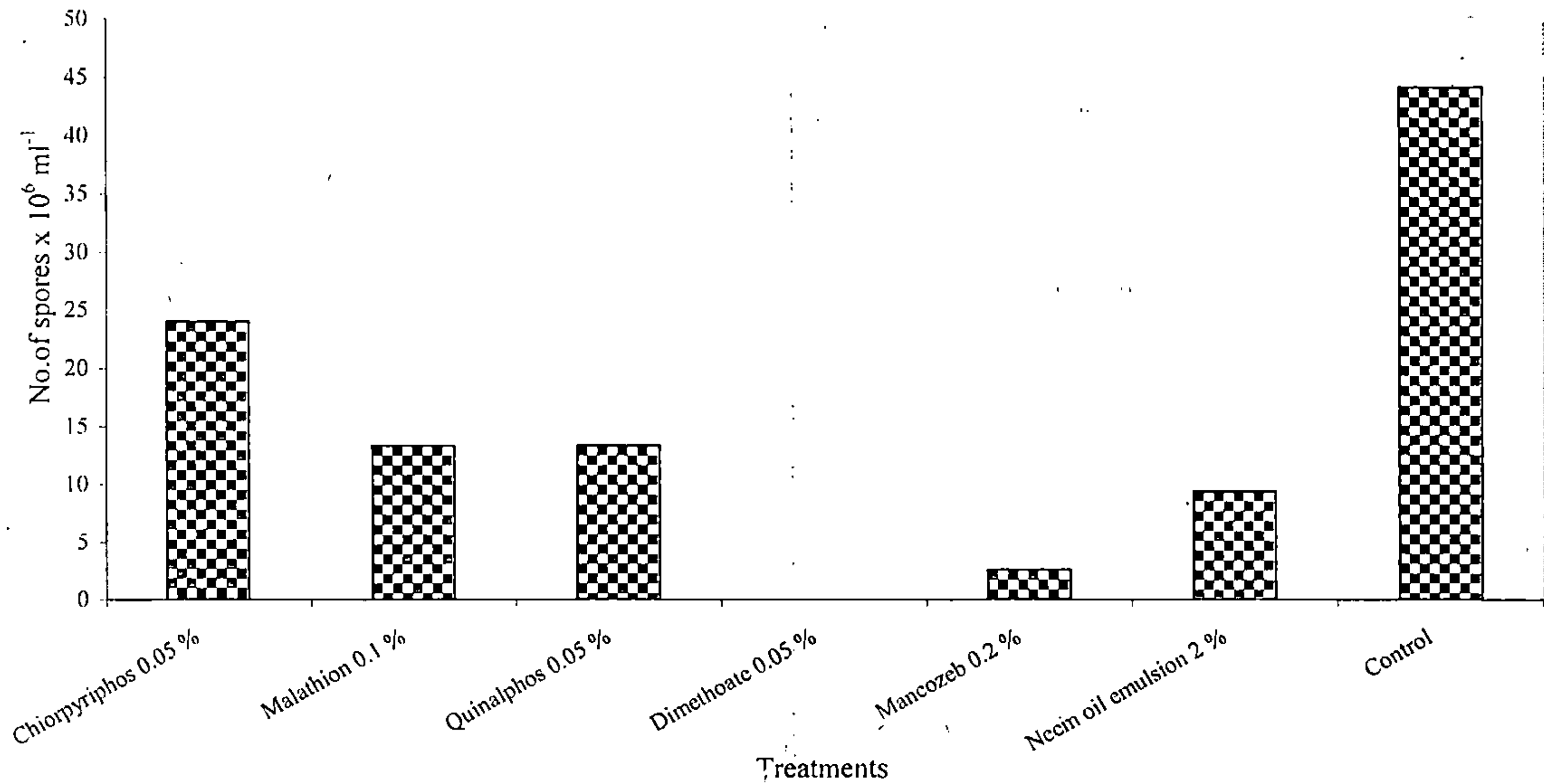


Fig. 11. Spore count (seven days after inoculation) of *F. pallidoroseum* on PDA poisoned with different pesticides

difference in the growth of the fungus grown in media containing chlorpyriphos (51.10) and malathion (52.35).

Significant difference in the per cent decrease in spore count was not noticed among the pesticides malathion, quinalphos, mancozeb and neem oil emulsion. Chlorpyriphos with 45.45 per cent caused least inhibition of sporulation.

4.5.3 *Rhizopus oryzae*

R. oryzae could cover the 9 cm petridish within three days of inoculation, in the control (Plate 10c). There was no inhibition of growth of the fungus when the media was incorporated with two per cent neem oil emulsion. All the other pesticides inhibited the growth of the fungus completely (Table 33 and Fig. 12). In the media containing neem oil emulsion, the fungus sporulated better than in control (3.96).

Table 33. Effect of different pesticides on mycelial growth and sporulation of *R. oryzae*

Sl.No.	Pesticides	% inhibition of mycelial growth	% increase or decrease of spore count
1.	Chlorpyriphos 0.05 %	100	-100
2.	Malathion 0.1 %	100	-100
3.	Quinalphos 0.05 %	100	-100
4.	Dimethoate 0.05 %	100	-100
5.	Mancozeb 0.2 %	100	-100
6.	Neem oil emulsion 2 %	0	+3.96

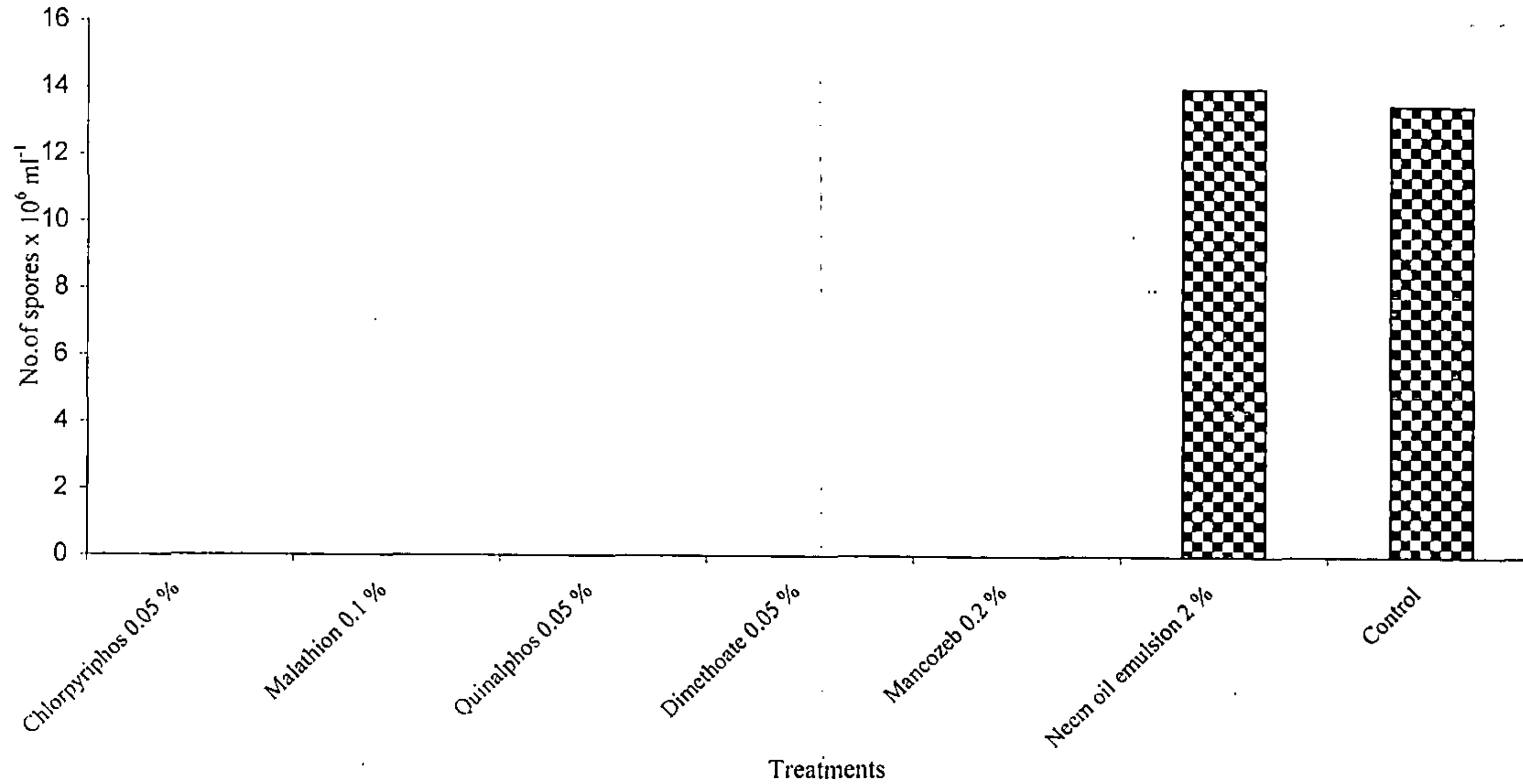
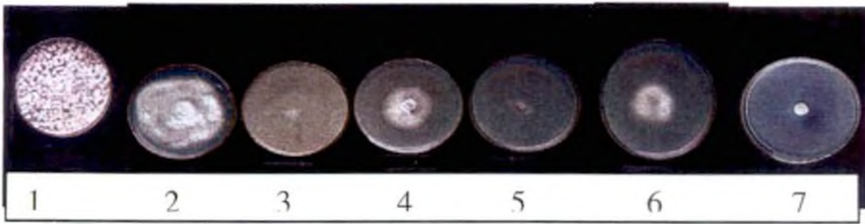
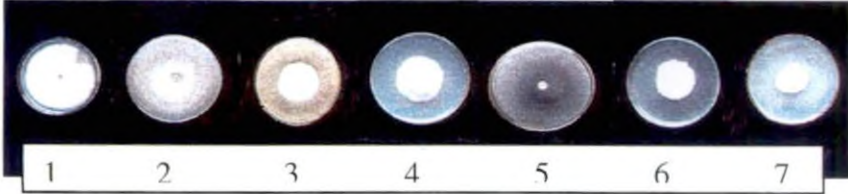


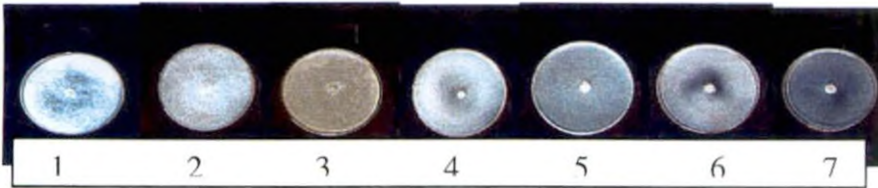
Fig. 12. Spore count (six days after inoculation) of *R. oryzae* on PDA poisoned with different pesticides



a. *B. bassiana*



b. *F. pallidoroseum*



c. *R. oryzae*

1 - Control
2 - Neem oil emulsion 2 %
3 - Mancozeb 0.2 %
4 - Chlorpyriphos 0.05 %
5 - Dimethoate 0.05 %
6 - Malathion 0.1 %
7 - Quinalphos 0.05 %

Plate 10. Growth of entomopathogenic fungi in media incorporated with various pesticides

DISCUSSION

5. DISCUSSION

Management of pests using microbial pathogens is generally a safer method compared to chemical method of pest control. Bacteria, fungi and viruses are the major entomopathogens, which are commercially utilized for insect pest management. Among these microbial pathogens, fungi are the only group, which can invade the insects actively through the cuticle. This is an advantage especially against the piercing and sucking insects. Since fungi act mainly by contact method, they can infect other non-target organisms like natural enemies and pollinators. But many workers have reported the species specificity of entomopathogenic fungi (Goettel *et al.*, 1990 and Hajek and Butler, 1999)

High humidity (75-85 %) and a temperature ranging from 20-35^{0}C noticed in Kerala, render a congenial atmosphere for the natural occurrence and multiplication of entomopathogenic fungi. The present study was conducted to find out the host range of seven commonly available entomopathogenic fungus among the vegetable pests in Kerala, and to identify suitable mass multiplication substrates for them.

Many entomopathogenic fungi appear to have a limited host range and are specific to single orders of arthropod pests, while others can infect insects belonging to different orders. For developing a mycoinsecticide, preliminary studies on the pathogenicity and host range of the fungus is important. The test fungus must be proved to be one infecting only harmful pests and not beneficial organisms before it is recommended for field application.

In the present study, isolates of seven commonly available entomopathgenic fungi *viz.*, *Beauveria bassiana, Beauveria brongniartii, Metarhizium anisopliae, Paecilomyces fumosoroseus, Paecilomyces lilacinus, Fusarium pallidoroseum* and *Rhizopus oryzae* were tested for

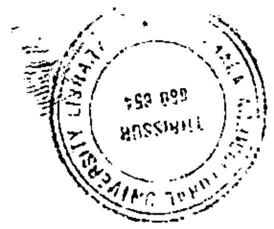
their pathogenicity to the sucking pests and leaf feeders of commonly cultivated vegetable crops of Kerala.

The isolate of *B.bassiana* under the present study was pathogenic only to the lepidopteran pests namely *Sylepta derogata, Spodoptera litura* and *Pericallia ricini*. It was not pathogenic to the other test insects including the lepidopteran pests like *Anadevidia peponis* and *Margaronia indica*.

In comparing the signs and symptoms, the fungal infected larvae resembled any other fungal infected lepidopteran larvae as described by Madelin (1966) and Ferron (1978). Infected larvae fed normally in the initial phase of the disease, but the appetite diminished 48 h after inoculation with the entomopathogenic fungus. The fungus usually takes some time to acclimatise with the environment and to multiply. During the 48 h period, the fungus gradually multiplies and cause infection of the insects. In the cultural studies conducted in the present trial (4.4) also the test fungi started to grow within 48 h. This initial period of time may be also the time taken by the fungus to surmount the insect's natural barriers to infection (Hall, 1976).

Between 48 and 72 hours after inoculation, the infected larvae become restless, unstable and ceased feeding. At death, the body became tough and mummified. Mycelial growth was visible all over the cadaver from 72 h after death. Similar symptoms were observed by Gopalakrishnan *et al.* (1999).

The isolate of *F. pallidoroseum* was found pathogenic to the aphids *A. craccivora* and *M. persicae*. The pathogencity of the fungus to these insects were earlier reported by Hareendranath (1989) and Rejirani (2001). Non-pathogenicity of *F. pallidoroseum* to other aphids like *Aphis malvae* was also reported by earlier workers. *F. pallidoroseum* was not infective to other test insects belong to Hemiptera, Coleoptera and Lepidoptera.



The development of mycoses observed in inoculated aphids was similar to earlier reports (Hareendranath, 1989; Fazial, 1992; Sunitha, 1997 and Rejirani, 2001). The affected aphids turned pale, sluggish and later turned brown. Growth of the fungus on the cadaver was visible as white mycelial mat.

Rhizopus oryzae used in the present study was found pathogenic to its original host *Dysdercus cingulatus* and was non-pathogenic to other insects tested.

All the three entomopathogenic fungi found effective in the present study were originally isolated from insects against which they are effective. *B. bassiana* was originally isolated from a lepidopteran pest of sugarcane and in the present study, it was found effective against the lepidopteran pests of vegetables.

The non-infectivity of the fungal isolates may be due to the specificity of the micro-organism. Burdeos and Villacarlos (1989) were of the opinion that the difference in virulence of the entomopathogenic fungi was affected by their specificity. According to them, pathogens that normally infect insects belonging to a particular order may be more virulent on species under the same order. There is a tendency for fungal isolates to have higher virulence to their original hosts or to closely related hosts than more distant relatives (Goettel *et al.*, 1990 and Hajek and Butler, 1999).

Resistance of the host insect to certain isolates of the pathogen may be another reason for the negative results of pathogenicity tests (Fargues, 1976 and Feng *et al.*, 1994). Often a strain presents no activity on a host while it causes a high mortality rate on other insects of the same family (Danfa and Valk, 1999).

The effectiveness of a fungal isolate is measured in terms of its pathogenicity and the speed with which it kills the target pest (Negasi *et al.*, 1998). In the present study a correlation between the doses of the

fungi and the responses of the test insects in terms of mortality was established and LC_{50} values were calculated using Probit Analysis.

Death of the 4th instar larvae of *S. derogata* inoculated with *B.bassiana* was noticed from the 4th day after inoculation onwards. Mortality rate was increased with the increase in the spore load. Fifty per cent mortality of the insects was noticed only from 8th day onwards, irrespective of the spore concentration tried. From 8th day onwards, those treatments with higher spore concentration gave a better death rate than those having low concentration. The LC_{50} values calculated for the 8th and 9th day after inoculation were 7.87×10^8 and 5.75×10^8 spores ml^{-1}.

When the spore concentration was below 5×10^8 ml^{-1}, even after the 9th day, the LC_{50} value was not reached. When the spore suspension was applied on the insects, the fungal spores germinated and started producing mycelia and new crop of spores. During this process, apart from directly affecting the metabolic process of the insects by drawing the nutrients, the fungi may also produce some toxins (Hall, 1976). For this a minimum spore load of the fungus might be required.

The fiducial limits calculated on the 8th and 9th day indicated that for 50 per cent mortality, the spore concentration of the inoculum should be in the range of 7.09×10^8 to 8.66×10^8 and 5.38×10^8 to 6.13×10^8 spores ml^{-1} respectively. These values agree with the result of laboratory experiment where 50 per cent mortality was observed on the 8th and 9th day when the spore concentration was between 5×10^8 and 1×10^9 spores ml^{-1}.

In the case of third instar larvae of *S. litura* inoculated with varying doses of *B. bassiana* 50 per cent mortality was observed only with the concentration above 1×10^9 spores ml^{-1}. The LC_{50} values calculated on the 5th and 6th day after inoculation was 9.11×10^8 and 6.99×10^8 spores ml^{-1}. The fiducial limits calculated on these days agree with the results of laboratory bioassays.

B. bassiana could cause more than 50 per cent mortality of third instar larvae of *P. ricini* at a lower concentration of 1.25 x 10^5 spores ml^{-1} and above, on the 4th day after inoculation. The LC_{50} values calculated for second and third day after inoculation were 5.56 x 10^5 and 2.72 x 10^5 spores ml^{-1}. From the value of fiducial limits calculated it is clear that for obtaining 50 per cent mortality of *P. ricini* larvae, the spore concentration should be in the range of 2.43 x 10^5 to 3.01 x 10^5 per ml.

Bioassay of *B. bassiana* based on dosage mortality relation was used by many previous workers to study the activity of the fungus on different insect pests (Brenes and Carballo, 1994, Ramkumar, 1998 and Khan and Gangapersad, 2001).

The different values of LC_{50} obtained in these studies may be due to the variation existed with reference to the isolate of the pathogen, the host, the stage of the host and the meteorological factors (Mathai, 1982).

Bioassays conducted with *F. pallidoroseum* to find out the LC_{50} of the fungus against *A. craccivora* and *M. persicae* obtained the values 3.86 x 10^6 and 0.72 x 10^6 spores ml^{-1} respectively. The fiducial limits obtained for 50 per cent mortality in the case of *A. craccivora* ranged from 3.78 x 10^6 to 3.95 x 10^6 spores ml^{-1} on the third day of inoculation. This finding is in accordance with the actual laboratory experiment. Hareendranath (1989) also observed similar values for *F. pallidoroseum* against *A. craccivora*.

In the case of *M. persicae* the fiducial limit for 50 per cent mortality ranged from 0.66 x10^6 to 0.78 x 10^6 spores ml^{-1}which is in accordance with the actual laboratory experiment.

Bioassay of *R. oryzae* on third instar nymphs of *D. cingulatus* obtained the LC_{50} values of 7.42 x 10^6, 6.34 x 10^6 and 4.15 x 10^6 spores ml^{-1} on the 5th, 6th and 7th day after inoculation with the fungus. Earlier work on the same fungus showed that at 5 x 10^6 spores ml^{-1} the fungus was pathogenic to the same pest (Mathai, 1999).

The approximate values of the slopes from bioassays conducted with the test fungi ranged from 1.2 to 6.4. Hall (1976) reviewed the slopes of several series of bioassays for a wide range of insect pathogens. Slopes were steeper (range 0.9 – 7.9) for assays involving the organism which produce toxins.

In any microbial control programmes, the development of mass production technique is important. In order to get better acceptability of biopesticide, it is necessary to reduce the cost of production of material. This is possible only by using low cost, locally available substances as carrier materials. The carrier material may vary from place to place depending upon the availability. In the present study, the suitability of locally available cheaper substrates, especially byproducts of agriculture were evaluated for the mass multiplication of entomopathogenic fungi.

The three entomopathogenic fungi tested (*B. bassiana, F. pallidoroseum* and *R. oryzae*) gave maximum growth and sporulation in rice bran and gingelly oil cake. It was found that the fungal growth was quick and complete in these two media.

Ferron (1981) reported solid substrate fermentation based on cereal grains such as rice as suitable for fungal pathogen. Hussey and Tinsley (1981) reported wheat bran as a suitable substrate for the mass production of *B. bassiana.* The growth and sporulation of *B. bassiana* was found maximum in rice based solid media by Ibrahim and Low, 1993; Vilas *et al.*, 1996 and Puzari *et al.*, 1997.

Rice bran as a suitable mass multiplication media for entomopathogenic fungi was earlier reported by Mathai *et al.* (1998), Faizal (1992), Anitha (2000) and Rejirani (2001). Higher nutrient status of rice bran [composition – carbohydrates 48.40 %, protein – 13.5 %, fat – 16.2 % and minerals – 6.6 % (Ca, P, Fe)] is the reason why it supported maximum fungal growth. Better growth and sporulation of the fungi in rice bran is due to the presence of nutrients in an available form.

Use of oil cakes for mass production of beneficial fungi was reported to be successful by Mani and Anandan (1989) ; Alam (1990). Rejirani (2001) reported that *F. pallidoroseum* grew and sporulated well on gingelly oil cake (composition – carbohydrate 25.0 %, protein – 18.30 %, fat 25.50 % and minerals – 5.2 %).

Among the liquid substrates tested for mass production (coconut water and starch solution), coconut water supported better growth of the fungi in the case of *B. bassiana, F. pallidoroseum* and *R. oryzae*. The number of spores produced was also maximum in coconut water. Coconut water [composition-carbohydrate – 4.4. %, protein – 1.4 %, fat – 0.1 % and minerals – 0.3 % (K, Mg, Cu, P)] is an ideal substrate for the fungus. This finding is in accordance with earlier works (Danger *et al.*, 1991, Manisegarame and Letchoumanane, 1996 and Rejirani, 2001). Thus it may be concluded that coconut water wasted from copra making industry may successfully be utilized for easy and economic multiplication of entomopathogenic fungi.

Different responses of the fungus in biomass and conidial production on different media may be related to the different nutritional composition of the media (Churchill, 1982 and Nina *et al.*, 1998). The nutrients present in the medium should be in the available form also. Apart from the nutrient status, an ideal substrate for fungal growth should have proper particle size and surface area to volume ratio.

A commercial insect control product cannot be expected to be kept under refrigeration to satisfy the requirements of the marketing system as the plant protection chemicals and other materials used for pest management by farmers are usually stored under room conditions (Couch and Ignoffo, 1981). Ferron (1981) considered short life of formulation as one of the factors which limits the use of microbial pesticides.

In the present study, the viability and virulence of *B. bassiana*, *F. pallidoroseum* and *R. oryzae* stored in rice bran, gingelly oil cake, coconut water and starch solution, showed a decrease due to storage at

room temperature. The liquid substrates did not retain the viability and virulence of the fungi, beyond two months. As suggested by Soper and Ward (1981) the loss of viability of fungus in liquid media may be due to the less stable nature of blastospores produced by liquid fermentation method.

The viability and virulence of *B.bassiana* in rice bran and gingelly oil cake was reduced drastically after three months of storage. Similar results were obtained by Clerck and Madelin (1965) and Walstad *et al.* (1970) where they observed the survival of *B. bassiana* spores for 0.5 months at 21^{0}C. Pereira and Roberts (1990) observed that dry mycelia of *B. bassiana* treated with maltose, sucrose and dextrose lost some of their conidial production capacity over a period of three months at room temperature. Rice bran and gingelly oil cake could not retain the virulence of the fungus after five months of storage at room temperature.

In the case of *F. pallidoroseum* stored in rice bran and gingelly oil cake, the virulence and viability was retained even at the end of six months, but the mortality per cent was below thirty. Similar results were obtained by Rejirani (2001) when the fungus was stored in charcoal, leaf mould and bran formulations.

Viability and virulence of spores present in formulated material depends upon the physical, chemical and biotic factors of the carrier material. The storage conditions were more critical to spore survival than the substrates used for spore production (Daoust and Roberts, 1983). Temperature fluctuations during the storage period was detrimental to the survival of the spores in the media.

R. oryzae stored in rice bran and gingelly oil cake retained the viability even after six months of storage, but the virulence of the fungus was lost after three months of storage. Several factors which operate during the extended storage period of the biopesticide will affect its survival. One of the major factors is the temperature fluctuations observed during the storage period. The range of temperature for

reproduction in fungus is comparatively much narrower than for growth. The spore production of the fungus used for the study is generally optimum between 25 - 28^0C (Bilgrami and Verma, 1978). Since the biopesticides were stored under fluctuating temperature condition the production and survival are affected. On the other hand when the fungi was stored under refrigerated condition, the survival was more (Sunitha, 1997) as the biological activity was curtailed to the minimum and the fungi were retained in a fixed temperature.

Integration of various pest control methods including microbial and chemical control methods is being promoted nowadays. The inhibitory or stimulatory effects of chemical pesticides on entomopathogenic fungi had been reported by many workers (Todorova *et al.*, 1998; Gupta *et al.*, 1999 and Gupta *et al.*, 2002). Compatibility of the commonly used chemical pesticides in vegetables, with *B. bassiana*, *F. pallidoroseum* and *R. oryzae* was analysed in the present study by conducting Poison Food Technique.

In the present study, out of the four insecticides, one fungicide and one botanical pesticide, inhibition was not noticed with *B. bassiana* and *R. oryzae* when two per cent neem oil emulsion was incorporated to the medium. Neem oil emulsion did not inhibit the colony growth and had least inhibition of sporulation in *B. bassiana* while the sporulation of *R. oryzae* was higher in the case of neem oil incorporated medium. In the case of *F. pallidoroseum*, neem oil supported the mycelial growth to certain extent but sporulation was highly reduced.

Gupta *et al.* (1999) reported stimulated growth of *B. bassiana* when neem leaf extract was incorporated to the growing medium. Increased efficacy of entomopathogenic fungi by mixing them with neem products had been reported by earlier workers (Babu *et al.*, 2000 and Sudharma and Hebsybai, 2000). Out of the four organophosphorus insecticides tried (chlorpyriphos, malathion, quinalphos and dimethoate) dimethoate completely inhibited the growth of all the test fungi.

Malathion, quinalphos and chlorpyriphos completely inhibited the growth of *R. oryzae*, while there was only partial inhibition of growth and sporulation of *B. bassiana* and *F. pallidoroseum*. Inhibitory action of organophosphorus compounds on fungi was recorded by Urs *et al.*, 1967; Easwaramoorthy and Jayaraj, 1978, Faizal, 1992 and Gupta *et al.*, 2002. But Kuruvilla and Jacob (1980) noticed less inhibitory action of certain organophosphorus insecticides on *Fusarium* spp. This indicate that variation in the inhibitory ability of organophosphorus insecticides on different fungi exists.

The fungicide, mancozeb inhibited the growth of *R. oryzae* completely and that of *B.bassiana* to an extent of more than 95 per cent. Eventhough 95 per cent inhibition of sporulation of *F. pallidoroseum* was observed in mancozeb incorporated medium, it reduced the mycelial growth only by 55.25 per cent. Todorova *et al.* (1988) also reported inhibition of mycelial growth and sporulation of *B.bassiana* by mancozeb.

Present observations coupled with the previous reports indicate that the fungus *B. bassiana* and *R. oryzae* could be integrated with neem oil emulsion for pest management programme.

SUMMARY

6. SUMMARY

The study on the "Effect of entomopathogenic fungi on sucking pests and leaf feeders of vegetables under *in vitro* conditions" was carried out in the Insect Pathology Laboratory of the College of Agriculture, Vellayani during 2000-2002.

Seven different entomopathogenic fungi *viz*., *Beauveria bassiana*, *Beauveria brongniartii*, *Paecilomyces fumosoroseus*, *Paecilomyces lilacinus*, *Metarhizium anisopliae*, *Fusarium pallidoroseum*, and *Rhizopus oryzae* were tested for their pathogenicity on ten sucking pests and six leaf feeders of vegetable crops. Out of these only three isolates namely *Beauveria bassiana*, *Fusarium pallidoroseum* and *Rhizopus oryzae* were found to be pathogenic to various test insects. *B. bassiana* was infective to *Sylepta derogata*, *Spodoptera litura* and *Pericallia ricini*, *F. pallidoroseum* to aphids, *Aphis craccivora* and *Myzus persicae* and *Rhizopus oryzae* to *Dysdercus cingulatus*.

Death of the larvae inoculated with *Beauveria bassiana* occurred from fourth day onwards in the case of *S. derogata* and *S. litura* while the third instar larvae of *P. ricini* died from 48 hours after infection. White powdery growth of the fungus appeared on the cadaver two to three days after death. *A. craccivora* and *M. persicae* infected with *F. pallidoroseum* died within two to three days after infection. The cadaver of the aphid was hard and mummified and found adhered to the host plant surface. Nymphs of *D. cingulatus* infected with *R. oryzae* died four to five days after infection and it sporulated within four to five days after death.

LC_{50} values were calculated for each fungus against different pests by carrying out bioassays. The LC_{50} value of *B. bassiana* against fourth instar larvae of *S. derogata* were 7.87×10^8 and 5.75×10^8 spores ml^{-1} on the eighth and nineth day after inoculation respectively. In the case of third instar larvae of *S. litura* 50 per cent mortality was obtained

when the spore concentration was 9.11×10^8 and 6.99×10^8 spores ml^{-1} on the fifth and sixth day after inoculation. The LC_{50} value of *B. bassiana* against third instar larvae of *P. ricini* was 5.56×10^5 and 2.72×10^5 spores/ml respectively on second and third day after inoculation. The LC_{50} value of *F. pallidoroseum* against the aphids *A. craccivora* and *M. perisicae* were 3.86×10^6 and 0.72×10^6 spores ml^{-1} respectively. For getting 50 per cent mortality of third instar nymphs of *D. cingulatus*, the concentrations of *R. oryzae* required was 7.42×10^6 spores ml^{-1} on the fifth day and 4.15×10^6 spores ml^{-1} on the 7th day after inoculation.

Mass multiplication of the fungi was carried out using different solid and liquid substrates. Rice bran and gingelly oil cake supported the growth of *B. bassiana, F. pallidoroseum* and *R. oryzae*. The three fungi grew well in coconut water. The sporulation of the fungi was better in rice bran compared to gingelly oil cake. The spore production in coconut water was comparatively lesser than that in solid media.

Shelf life of the three-entomopathogenic fungi *viz.*, *B. bassiana*, *F. pallidoroseum* and *R. oryzae* grown in rice bran, gingelly oil cake, coconut water and starch solution and stored at room temperature was studied for a period of six months. The liquid media (coconut water and starch solution) could not retain the viability and virulence of the fungi beyond two months of storage. The sporulation of fungi in different media also showed a gradual reduction with period of storage.

The viability of *B. bassiana* stored in rice bran and gingelly oil cake was retained till the end of five months of storage. But the virulence of *B. bassiana* was drastically reduced after three months of storage and was completely lost at the end of six months.

F. pallidoroseum showed better shelf life in the solid substrate (rice bran and gingelly oilcake) and they could retain the viability and virulence of the fungus even at the end of six months. But there was considerable reduction in the viability and virulence of *F. pallidoroseum* after four months of storage.

R. oryzae stored in rice bran and gingelly oil cake did not retain the virulence at the end of fourth month of storage. But the fungus was viable even at the end of six months.

Compatibility studies with six commonly used pesticides, showed that neem oil emulsion at two per cent concentration did not inhibit the growth and sporulation of *B. bassiana* and *R. oryzae*, but it inhibited the growth of *F. pallidoroseum*. The growth of *B. bassiana* and *R. oryzae* was completely inhibited by dimethoate and mancozeb, but mancozeb allowed some mycelial growth of *F. pallidoroseum*. All other insecticides inhibited the growth of the fungi in different proportions.

REFERENCES

7. REFERENCES

Aguda, R. M., Romback, M. C. and Robert, D. W. 1988. Effect of pesticides on germination and growth of three fungi of rice insects. *Int. Rice Res. Newsl.* 13: 39 – 40

Alam, M. M. 1990. *Paecilomyces lilacinus*: A nematode biocontrol agent. *Nematode Bio Control: Aspects and Prospects*. (eds.. Jairajapuri, M. S., Alam, M. M. and Ahmad, L.). CBS Publishers and Distributors, Delhi, pp. 71 - 82

*Alvarez, M. I. G., Rivero, L. F. V. and Cotes, A. M. 1997. Massive production and preformulation of *Metarhizium* spp. for Ilanera locust biological control. *Revista Colombiana de Entomologia* 23: 3 – 4

Anderson, T. E., Hajek, A. E., Roberts, D. W., Presler, H. K. and Robertson, J. L. 1989. Colorado potato beetle coleoptera: chrysomelidae : Effects of combination of *B. bassiana* with insecticides. *J. econ. Ent.* 82: 83 – 89

Anderson, T. E. and Roberts, D. W. 1983. Compatibility of *B.bassiana* isolates with insecticide formulations used in Colorado potato beetle (Coleoptera: Chrysomelidae) control. *J. econ. Ent.* 76: 1437 – 1441

Anitha, N. 2000. Bioecology and integrated management of banana pseudostem weevil *Odoiporus longicollis* Oliv. Ph.D thesis, Kerala Agricultural University, Thrissur, p. 178

Aregger, E. 1992. Conidia production of the fungus *Beauveria brongniartii* on barley and quality evaluation during storage at 2^{0}C. *J. Invertebr. Pathol.* 59: 2 – 10

Babu, R., Murugan, K., Sivaramakrishnan, S. and Thiagarajan, P. 2000. Efficacy of Neem and entomopathogenic fungus, *Beauveria bassiana* on *Spodoptera litura* Fb. Entomocongress 2000. Perspectives for the new millennium, 5 – 8 November 2000, Association for Advancement of Entomology, Trivandrum, Kerala. *Abstract* 6

*Badilla, F. F. and Alves, S. B. 1991. Control of sugarcane weevil *Sphenophorus levis* with *Beauveria* spp. under laboratory and field conditions. *Anais da Sociedade Entomologica do Brasil* 20: 251 – 263

*Bajan, C. and Kmitowa, K. 1969. Pathogenicity of entomogenous fungi isolated from hibernating imagines of the colorado beetle *Leptinotarsa decemlineata*. *Ekol. Pol.* 17: 409 – 420

Bassi, A. 1836. *Del Mal del Segno Calcinaccio o Moscardino*. Parte Seconda, Practica, Lodi, Orcesi, p. 1 – 67

Batista, F. A., Camargo, L. M. P. C. A., Myazaki, I., Cruz, B. P. B. and Olivera, D. A. 1989. Biological control of the banana root borer (*Cosmopolites sordidus* Germer) by entomogenous fungi in the laboratory. *Biologico* 53: 1 – 6

Beevi, N. S. 1979. Studies on entomogenous fungi associated with lady bird beetle *Henosepilachna vigintioctopuncta* (Fabr.) (Coccinellidae:Coleoptera). M.Sc.(Ag.) thesis, Kerala Agricultural University, Thrissur, p. 72

Beevi, N. S. 1982. Susceptibility of different pests and plants to infections of *Fusarium moniliformae* var. *subglutinans*. *Entomon* 7: 235 – 236

Bilgrami, K. S. and Verma, R. N. 1978. *Physiology of fungi.* Second edition, Vikas Publishing House Pvt. Ltd., New Delhi, p. 507

Blachere, H., Calvez, J. and Ferron, P. 1973. Etude de la formulation et de la conservation d' une preparation entomopathogenic a base de blastospores de *Beauveria tenella* (Delacr.Siemaszko). *Ann. Zool. Ecol. Anim.* 5: 69 – 79

Brandenberg, R. L. and Kennedy, G. G. 1983. Interactive effects of selected pesticides on the two spotted spider mite and its fungal pathogen *Neozygites floridana. Ent. Exp. Applic.* 34: 240 – 244

*Brenes, S. and Carballo, V. M. 1994. Evaluation of *Beauveria bassiana* for the biological control of the plantain weevil *Cosmopolites sordidus. Manejo-Integrado-de plagas* 31: 17 – 21

Burdeos, A. T. and Villacarlos, L. T. 1989. Comparative pathogenicity of *Metarhizium anisopliae, Beauveria bassiana* and *Paecilomyces lilacinus* to adult sweet potato weevil, *Cylas formicarius* (F.) (Coleoptera: Curculionidae). *Philipp. Ent.* 7: 561 – 571

Chabchoul, H. and Taborsky, V. 1991. Use of *Metarhizium anisopliae* (Metch) Sorokin against Colorado beetles. *Agricultura Tropica et Subtropica* 24: 31 – 38

Charnley, A. K. 1989. Mycoinsecticides : Present use and future prospects. Progress and prospects in insect control. *British Crop Protection Council monograph* 43: 165 – 181

Chaudhuri, S. 1976. *Metarhizium anisopliae* (Metch) Sorokin on brinjal mealy bug and its use in biological control of the pest. *Curr. Sci.* 45: 34

Chaudhuri, D., Kanaujia, K. R. and Rathore, R. R. S. 2001. Mycopesticidal dust formulations: evaluation of diluants for viability of *Beauveria bassiana* (Balsamo) Vuillemin. *Proceedings of Symposium on biocontrol based pest management for quality crop protection in the current millennium, July 18 – 19 2001* (eds. Singh, D., Mahal, M. S., Sohi, A. S., Dilawari, V. K., Brar, K. S. and Singh, S. P.). Punjab Agricultural University, Ludhiana, pp. 87 – 88

*Chen, Z., Huang, J. R., Xu, Y. W. and Yao, L. J. 1990. Field experiment on the pathogenicity of *Paecilomyces cicada* to *Pieris rapae*. *Chin. J. Biol. Control* 6: 131 – 133

*Chen, Z., Xie, P. H., Huang, J. R., Pan, L. C., Feng, H. G. and Zhang, A .W. 1995. Infectivity of *Metarhizium anisopliae* against the mulberry brown chaffer *Holotrichia paralella*. *Chin. J. Biol. Control* 11: 54 – 55

*Churchill, B. W. 1982. Mass production of microorganism for biological control. *Biological Control of Weeds with Plant Pathogen*. (eds. Chruduttan, R. and Walker, H. L.). John Wiley and Sons, New York, pp. 139 – 156

Clerck, G. C. and Madelin, M. F. 1965. The longivity of conidia of three insect parasitizing hyphomycetes. *Trans. Brit. Mycol. Soc.* 48: 193 – 209

*Couch, T. L. and Ignoffo, C. M. 1981. Formulation of insect pathogens. *Microbial control of pests and plant diseases 1970 - 1980* (ed. Burges, H. D.). Academic Press, New York, pp.621 – 634

Danfa, A. and Valk, H. C. H. G. 1999. Laboratory testing of *Metarhizium* spp. and *Beauveria bassiana* on Sahelian non-target arthropods. *Biocontrol Sci. Technol.* 9: 187 – 198

Danger, T. K., Geetha, L., Jayapal, S. P. and Pillai, G. B. 1991. Mass production of entomopathogen *Metarhizium anisopliae* in coconut water wasted from copra making industry. *J. Plantn. Crops* 19: 54 – 69

Daoust, R. A. and Roberts, D. W. 1983. Studies on the prolonged storage of *Metarhizium anisopliae* conidia: Effect of growth substrate on conidial survival and virulence against mosquitoes. *J. Invertebr. Pathol.* 41: 161 – 170

Dara, S. K. and Hountondji, F. C. C. 2001. Effects of formulated imidacloprid on two mite pathogens, *Neozygites floridana* (Zygomycotina : Zygomycetes) and *Hirsutella thompsonii* (Deuteromycotina : Hyphomycetes). *Insect Sci. Applic.* 21: 133 – 138

Diaz, S., Grillo, J. and Ravelo, H. 1986. An isolate of *Beauveria bassiana* as a pathogen of *Cylas formicarius*. *Centro Agricola* 13: 94 – 95

Dorschner, K. W., Feng, M. G. and Baird, C. R. 1991. Virulence of an aphid derived isolate of *Beauveria bassiana* to hop aphid, *Phorodon humuli* (F. Aphididae). *Environ. Ent.* 20: 690 – 693

Easwaramoorthy, S. and Jayaraj, S. 1978. Effectiveness the white Halo fungus *Cephalosporium lecanii* against field population of coffee greenbug *Coccus viridis*. *J. Invertebr. Pathol.* 32: 88 – 96

Eayre, C. G., Jafee, B. A. and Zehr, E. I. 1990. Influence of potassium on spore germination in the nematophagous fungus *Hirsutella rhossiliensis*. *J. Nematol.* 22: 612 – 613

Ekesi, S. 2000. Pathogenicity and antifeedant activity of entomopathogenic *Hyphomycetes* to the cowpea leaf beetle *Ootheca mutabilis* Shalberg. *Insect Sci. Applic.* 21: 55 – 60

Ekesi, S., Maniania, N. K., Onu, I. and Lohr, B. 1998. Pathogenicity of entomopathogenic fungi (Hyphomycetes) to the legume flower thrips *Megalurothrips sjostedi*. *J. Appl. Ent.* 122: 629 – 634

Faizal, M. H. 1992. Studies on the entomogenous fungus *Fusarium pallidoroseum* (Cooke.) *Sacc.* associated with cowpea aphid *Aphis craccivora* Koch. M.Sc.(Ag.) thesis. Kerala Agricultural University, Thrissur, p. 83

Faizal, M. H. and Mathai, S. 1997. Effect of pesticides on the growth and sporulation of entomogenous fungus *Fusarium pallidoroseum*. *Indian J. Ent.* 59: 135 – 140

Fargues, J. 1976. Specificite des champignons pathogens imparfaits (Hyphomycetes) pour les larves de coleopteres (Scarabaeidae et chrysomelidae). *Entomophaga* 21: 314 – 323

Feng, M. G., Poprawski, T. J. and Khachatourians, G. G. 1994. Production, formulation and application of the entomopathogenic fungus *Beauveria bassiana* for insect control: current status. *Biocontrol Sci. Technol.* 4: 3 – 34

Ferron, P. 1978. Biological control of insect pests by entomogenous fungi. *Ann. Rev. Ent.* 23: 409 – 442

Ferron, P. 1981. Pest control by the fungi *Beauveria* and *Metarhizium* in microbial control of pests and diseases. (ed. Burges, H. D.). Academic Press, London, pp. 465 – 483

Finney, D. J. 1952. *Probit Analysis – a statistical treatment of the sigmoid response curve.* Second edition. University Press, Cambridge, p. 318

Gindin, G., Geschtovt, N. U., Raccah, B. and Barash, I. 2000. Pathogenicity of *Verticillium lecanii* to different developmental stages of the silver leaf whitefly, *Bemisia argentifolii*. *Phytoparasitica* 28: 229 – 239

Goettel, M. S., Poprawski, T. J., Vandenberg, J. D., Li, Z. and Roberts, D. W. 1990. Safety to non-target invertebrates of fungal biocontrol agents. *Safety of Microbial Insecticides* (eds. Lard, M., Lacey, L. A. and Davidson, E. W.). CRC Press, Boca Raton, FL, USA, pp. 209 – 231

Gopalakrishnan, C., Anusuya, D. and Narayanan, K. 1999. Occurrence of entompathogenic fungi *Paecilomyces farinosus* (Holmskiold) Brown and Smith and *Zoophthora radicans* (Brefeld) Batko on the field population of *Plutella xylostella* L. on cabbage. *Entomon* 24: 363 – 369

Gopalakrishnan, C. and Mohan, K. S. 2000. Effect of certain insecticides and fungicides on the conidial germination of *Nomuraea rileyi* (Farlow) Samson. *Entomon* 25: 217 – 223

Gopalakrishnan, C. and Narayanan, K. 1988. Occurrence of two entomofungal pathogens *Metarhizium anisopliae* and *Nomuraea rileyi* on *Heliothis armigera*. *Curr. Sci.* 57: 867 – 868

Gopinathan, P. B., Beevi, N. S. and Nair, M. R. G. K. 1982. Occurrence of *Fusarium equiseti* (corda) Sacci as a fungal pathogen of brinjal mealy bug *Coccidohystrix insolita* (Green). *Entomon* 7: 120

*Grunberg, M., Adam, H., Walter, C. and Hirte, W. F. 1988. Possibilities for using the entomopathogenic fungus *Verticillium lecanii* for the biological control of aphids in crops under glass and plastic. *Nachrichtenblefflur den pflanzenschutz in des* 42: 186 – 190

Gupta, P., Paul, M. S. and Sharma, S. N. 1999. Studies on compatibility of white muscardine fungus *Beauveria bassiana* with some neem products. *Indian Phytopath.* 52: 278 – 280

Gupta, R. B. L., Sharma, S. and Yadava, C. P. S. 2002. Compatibility of two entomofungi *Metarhizium anisopliae* and *Beauveria bassiana* with certain fungicides, insecticides and organic manures. *Indian J. Ent.* 64 : 48 – 52

Hajek, A. E. and Butler, L. 1999. Predicting the host range of entomopathogenic fungi. *Non-target Effects of Biological Control* (eds. Follett, P. A. and Duan, J. J.). Kluwer Academic, Netherlands, pp. 95 - 108

Hall, R. A. 1976. A bioassay of the pathogenicity of *Verticillium lecanii* conidiospores on the aphid *Macrosiphoniella sanborni*. *J. Invetebr. Pathol.* 27: 41 – 48

Hall, R. A. 1982. Control of the whitefly, *Trialeurodes varporariorum* and cotton aphid *Aphis gossypii* in glasshouse by two isolates of the fungus *Verticillium lecanii*. *Ann. Appl. Biol.* 10: 1 – 11

Haraprasad, N., Niranjana, S. R. and Shetty, H. S. 2001. Mass production on solid substrates and enhancement of conidiation of

Beauveria bassiana for control of coffee berry borer. *Proceedings on Symposium on Biocontrol Based Pest Management for Quality Crop Protection in the Current Millennium, July 18 – 19, 2001* (eds. Singh, D., Mahal, M. S., Sohi, A. S., Dilawari, V. K., Brar, K. S. and Singh, S. P.). Punjab Agricultural University, Ludhiana, p. 132 – 133

Hareendranath, V. 1989. Control of *Aphis craccivora* Koch. with fungal pathogens and their impact on the natural enemies of the pest. M.Sc.(Ag.) thesis, Kerala Agricultural University, Thrissur, p. 87

Hareendranath, V., Nair, P. K. V. and Paulose, S. 1987. *Fusarium pallidoroseum* (cooke) Sacc. as a fungal pathogen of *Aphis craccivora* Koch. *Entomon* 12: 392 – 394

*Hassani, M., Zimmermann. G. and Vidal, S. 2000. *Paecilomyces fumosoroseus*: efficacy against the cotton pests *Spodoptera littoralis* (Lepidoptera : Noctuidae), *Helicoverpa armigera* (Lepidoptera : Noctuidae) and *Aphis gossypii* (Homoptera : Aphididae) and effect of temperature on germination and mycelial growth. *Proceedings of the seventh European meeting of the International Organisation for Biological Control / West Palearctic Regional Section working group: Insect pathogens and insect parasitic nematodes entitled capturing the potential of biological control, March 22 – 26, 1999* (ed. Smith, P. H.). International Organisation for Biological Control, New York, p. 217 – 221

Hebsybai, Mathai, S. and Gokulapalan, C. 1999. *Rhizopus oryzae* Went and Gerlings, a new fungal pathogen of *Dysdercus cingulatus* (F.). *Insect Environ.* 4: 129 – 130

Hussey, N. W. and Tinsley, J. W. 1981. Improvement of insect pathology in the peoples Republic of China. *Microbial Control of Pests and Plant Diseases*. (ed. Burges, H. D.). Academic Press, New York, pp. 785 – 796

Ibrahim, Y. B. and Low, W. 1993. Potential of mass production and field efficacy of isolates of the entomopathogenic fungi *Beauveria bassiana* and *Paecilomyces fumosoroseus* against *Plutella xylostella*. *Int. J. Pest. Mgmt.* 39: 288 – 292

Ignoffo, C. M. 1978. Strategies to increase the use of entomopathogens. *J. Invertebr. Pathol.* 31: 1 – 3

Ignoffo, C. M., Garcia, C. and Kruka, M. 1983. A leaf surface treatment bioassay for determining activity of conidia of *Beauveria bassiana* against *Leptinotarsa decemlineata*. *J. Invertebr. Pathol.* 41: 385 –386

Ignoffo, C. M., Hostetter, D. L., Garcia, C. and Pinnel, R. E. 1975. Sensitivity of the entomo-pathogenic fungus *Nomuraea rileyi* to chemical pesticides used in soybeans. *Environ. Ent.* 4: 765 – 768

Inglis, G. D., Feniuk, R. P., Goettel, M. S. and Johnson, D. L. 1995. Mortality of grasshoppers exposed to *Beauveria bassiana* during oviposition and nymphal emergence. *J. Invertebr. Pathol.* 65: 139 – 146

Jacob, A., Kuruvilla, S., Philip, B. M. and Asari, P. A. R. 1978. *Fusarium moniliformae* var. *subglutinans* Wollenw and Reink. pathogenic to the spotted beetle *Epilachna vigintioctopunctata*. *Agric. Res. J. Kerala*. 16: 262 – 263

Jenkins, N. E., Heviefo, G., Langewald, J., Cherry, A. J. and Lomer, C. J. 1998. Development of mass production technology for aerial

conidia for use as mycopesticides. *Biocontrol News Inf.* 19: 21 – 31

Jenkins, N. E. and Prior, C. 1993. Growth and formulation of true conidia by *Metarhizium flavoviridae* in a simple liquid medium. *Mycol. Res.* 97: 1489 – 1494

Johnson, D. L. and Goettel, M. S. 1993. Reduction of grasshopper populations following field application of the fungus *Beauveria bassiana. Biocontrol Sci.Technol.* 3: 165 – 167

Khan, A. and Gangapersad, G. 2001. Comparison of the effectiveness of three entomopathogenic fungi in the management of the banana bore weevil, *Cosmopolites sordidus* (Germar) (Coleopter: Cuurculionidae). *Int. Pest Control* 43: 208-213

Klochko, M. D. 1969. The use of entomopathogenic fungi for controlling *Epilachna vigintioctopunctata. Inst. Zashch. Rast.* 1: 26 – 31

*Kunckel d' Hercularis, J. and Langlois, C. 1891. Less champigons parasites des Acridiens. *Conspt. Rend. Acad. Sci.* 112: 1465 – 1468

Kuruvilla, S. and Jacob, A. 1980. Pathogenicity of the entomogenous fungus *Paecilomyces farinosus* (Dickson ex Fries) to several insect pests. *Entomon* 5: 175 – 178

Lacey, L.A. and Fransen, J. J. 1994. Fungi as biological control agents of *Bemisia tabaci. Bemisia Newsl.* 8: 34 – 35

Madelin. 1966. Fungal parasites of insects. *Ann. Rev. Ent.* 11: 423 – 448

Mani, A. and Anandan, R. J. 1989. Evaluation of plant leaves, oil cakes and agro industrial wastes as substrates for mass multiplication of

nematophagous fungus *Paecilomyces lilacinus*. *J. Biol. Control*. 3: 56 – 58

Manisegarame, S. and Letchoumanane, S. 1996. *Fusarium pallidoroseum* a pathogen on the rice leaf roller *Cnaphalocrosis medinalis*. *Indian J. Ent.* 58: 364 – 368

Masuda, T. 2000. Microbial control of the DBM *Plutella xylostella*, by an entomopathogenic fungus, *Beauveria bassiana*. Effects of temperature on mycoses and conidial invasion time. *J. Appl. Ent. Zool.* 44: 177 – 182

Mathai, S. 1982. Joint action of insect pathogen insecticide mixtures in the control of crop pests. Ph.D thesis, Kerala Agricultural University, Thrissur, p. 94

Mathai, S. 1999. Host range of a potent entomopathogenic fungus *Rhizopus oryzae* Went and Gerlings. *Insect Environ.* 4: 129 – 130

Mathai, S., Geetha, D. and Mohandas, N. 1998. Use of different locally available and cheaper substrates for mass multiplication of *Fusarium pallidoroseum* (Cooke) Sacc. an effective fungal pathogen against the pea aphid *Aphis craccivora* Koch. New Trends in Biotechnology, 1992 (eds. Subbarao, N. S., Balagopalan, C and Ramakrishnan, S.V). pp. 249 – 251

Meade, D. L. and Byrne, D. N. 1991. The use of *Verticillium lecanii*, against subimaginal instars of *Bemisia tabaci*. *J. Invertebr. Pathol.* 57: 296 – 298

Meadow, R., Vandenbery, J. Shelton, D. A. M. 2000. Exchange of inoculum of *Beauveria bassiana* (Bals.) Vuill. (Hyphomycetes) between adult flies of the cabbage maggot *Delia radicum* L. (Diptera: Anthomyiidae). *Biocontrol Sci. Technol.* 10: 479 – 485

*Metchnikoff, E. 1879. Diseases of the larvae of the grain weevil. *Insects Harmful to Agriculture*. Odessa Zemstvo office, Odessa, p. 32

Miranpux, G. S. and Khachatourians, G. G. 1993. Application of entomopathogenic fungus *Beauveria bassiana* against green peach aphid *Myzus persicae* infesting canola. *J. Insect Sci.* 6: 287 – 289

Moore, D., Bateman, R. P., Carey, N. and Prior, C. 1995. Long term storage of *Metarhizium flavoviridae* conidia in oil formulations for the control of locusts and grasshoppers. *Biocontrol Sci. Technol.* 5: 193 – 199

Moore, D. and Prior, C. 1993. The potential of mycoinsecticides: *Biocontrol News Inf.* 14: 31 – 40

Nagalingam, B. 1983. Studies on the ecology and biocontrol agents of *Myzus persiae* (Sulzer) Ph.D. thesis, Tamil Nadu Agricultural University, Coimbatore, p. 102

Nagalingam, B. and Jayaraj, S. 1986. First record of *Fusarium semitectum* Berk and Rav as an entomophagous fungus. *Curr. Sci.* 55 : 377 - 388

Narayanasamy, P. 1998. Mycoparasites check rice brown plant hopper (BPH) in India. *Insect Enviro.* 4: 12

Narvaez, G., Del, M. P., Cronzalez, G. M. T., Bustillo, P. A. E., Chaves, C. and Montoya, R. E. C. 1997. Spore production of *Beauveria bassiana* and *Metarhizium anisopliae* isolates in different substrates. *Revista Colombiana de Entomologia* 23: 125 – 131

67

Negasi, A., Parker, B. L. and Brownbridge, M 1998 Screening and bioassay of entomopathogenic fungi for the control of silverleaf whitefly *Bemisia argentifolii. Insect Sci. Applic.* 18: 37 – 44

Nehru, C. R. and Jayarathnam, K. 1993. Evaluation of biological and chemical control strategies against the white grub (*Holotrichia serrata*) infesting rubber seedlings. *Indian J. Natural Rubb. Res.*6: 159 - 162

Nene, Y. L. 1973. Note on a fungus parasite of *Bemisia tabaci* Genn. a vector of several plant viruses. *Indian J. Agric. Sci.* 43: 514 – 516

Nina, J. E., Heviefo, G., Langewald, J., Andrew, J. C. and Lomer, C. J. 1998. Development of mass production technology for aerial conidia for use as mycopesticides. *Biocontrol News Inf.* 19: 21 – 31

*Orozco, M., Farias-Larios, J., Lopez-Perez, J. and Ramirez, N. R. 2000. Use of *Beauveria bassiana* for the control of *Bemisia argentifolii* in melon. *Manejo Integrado de Plagas* 56: 45 - 51

Olson, D. L. and Oetting, R. D. 1999. The efficacy of mycoinsecticides of *Beauveria bassiana* against silverleaf whitefly (Homoptera: Aleyrodidae) on poinsettia. *J. agric.Urban Ent.* 16: 279 – 185

Padmaja, V. and Kaur, G. 1998. Relative susceptibility of brinjal spotted beetle (*Henosepilachna vigintioctopunctata*) to certain isolates of *Beauveria bassiana* (Bals). Vuill. *J. Biol. Control.* 12: 147 – 151

Pereira, R. M. and Roberts, D. W. 1990. Dry mycelium preparations of entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana. J. Invertebr. Pathol.* 56: 39 – 46

Poprawski, T. J. and Khachatourians, G. G. 1994. Production, formulation and application of the entomopathogenic fungus *Beauveria bassiana* for insect control: current status. *Biocontrol Sci. Technol.* 4: 3 – 34

Puzari, K. C., Surmath, D. K. and Hazariku, L. K. 1997. Medium for mass prduction of *Beauveria bassiana* (Balsamo) Vuillemin. *J. Biol. Control* 11: 97 – 100

Ramarethinam, S., Loganathan, S., Marimuthu, S. and Murugesan, N. V. 2001. Pathogencity of the entomopathogenic fungus, *Paecilomyces fumosoroseus* (Dueteromycotina : Hyphomycetes) on yellow mite, *Polyphagotarsonemus latus* (Banks) (Bcar: Tarsonomidae) infesting cotton plants in South India. *Pestology* 4: 1 – 6

Ramaseshiah. 1967. The fungus *Entomophthora coronata* parasitic on three species of aphids infesting crucifers in India. *J. Inverterbr Pathol.* 9: 128 – 130

*Ramisch, I. 1976. *Paecilomyces farinosus* as a parasite of potato beetle (*Leptinotarsa decemlineata*). *Nova Hedwigia.* 27 : 199 – 214

Ramkumar. 1998. Studies on integration of entomopathogens for the management of *Spodoptera litura* (Fabricius) (Lep.) on cabbage. M.Sc.(Ag) Thesis. University of Agricultural Sciences, Bangalore, p. 110

*Ramos, E. Q., Alves, S. B., Tanzin, M. R. and Lopes, R. B. 2000. Susceptibility of *Bemisia tabaci* to *Beauveria bassiana* under laboratory conditions. *Manejo Integrado de plagas* 56: 65 – 69

Rana, R. L. and Villacarlos, L. T. 1991. Effect of *Metarhizium anisopliae* (Metch.) Sorokin infection on the fecundity and

survival of the sweet potato weevil *Cylas formicarius* (Fabr.) (Coleoptera: Curculionidae). *Philipp. Ent.* 8: 963 – 972

Rejirani, O. P. 2001. Production and evaluation of the fungus *Fusarium pallidoroseum* (Cooke) Sacc. as a biopesticide against pea aphid *Aphis craccivora* Koch. PhD thesis, Kerala Agricultural University, Thrissur p. 145

Sapieha, A. and Mietkiewski, R. 1992. The influence of chitin synthesis inhibitor on growth of entomopathogenic fungi *in vitro*. *Acta Mycologica*. 27 : 189 – 195

Saxena, H. and Ahmad, R. 1997. Field evaluation of *Beauveria bassiana* (Balsamo) Vuillemin against *Helicoverpa armigera* (Hubner) infecting chickpea. *J. Biol. Control.* 11: 93 – 96

Selvasundaram, R. and Muraleedhran, N. 2000. Occurrence of the entomogenous fungus *Beauveria bassiana* on the short hole borer of tea. *J. Plantn. Crops* 28: 229 – 230

Shaw, M. W. 1959. The diamond back moth *Plutella maculipennis* (Curtis). A historical review with special reference to its occurrence in Scotland in 1958. *Trans. R. Highl. Agric. Soc.* 6: 56 – 80

Soper, R. S. and Ward, M. G. 1981. Production, formulation and application of fungi for insect control. *Biological Control in Crop Production* (ed. Papavizas, G.C.). Allanheld Press, New Jersey, pp. 161 – 180

Sopp, P. I., Gillespine, A. T. and Palmer, A. 1989. Application of *Verticillium lecanii* for the control of *Aphis gossypii* by a low volume electrostatic rotary atomizer and a high volume hydraulic sprayer. *Entomophaga* 34: 417 – 428

Strawinski, K. 1930. Cabbage butterfly: biology and control. *Poisk. Pirmo. Ent.* 8: 227 – 248

Sudharma, K. and Hebsybai. 2000. Utilization of biopesticides for the management of pea aphid *Aphis craccivora* Koch. Entomocongress 2000. Perspectives for the new millennium, 5 – 8 November 2000, Association for Advancement of Entomology, Trivandrum, Kerala. *Abstract* 14

Sunitha, V. S. 1997. *Fusarium pallidoroseum* (Cooke) Sacc as a biocontrol agent for the pea aphid *Aphis craccivora* Koch. M.Sc.(Ag.) Thesis, Kerala Agricultural University, Thrissur, p. 76

Todorova, S. I., Coderre, D., Duchesne, R. M. and Cote, J. C. 1998. Compatibility of *Beauveria bassiana* with selected fungicides and herbicides. *Environ. Ent.* 27 : 427 – 433

Urs, R. N. V., Govindu, H. C. and Shivashankarashastry, K. S. 1967. The effect of certain insecticides on the entomogenous fungi *Beauveria bassiana* and *Metarhizium anisopliae*. *J. Invertebr. Pathol.* 9: 398 – 403

Vanninen, L., Hokkanen, H. and Tyni – Juslin, J. 1999. Attempts to control cabbage root flies *Delia radicum* L. and *Delia floralis* (Fall.) (Dipt: Anthomyiidae) with entomopathogenic fungi: laboratory and greenhouse tests. *J. Appl. Ent.* 123: 107 – 113

Vilas, B. A. M., Andrado, R. M. and Oliverira, J. V. 1996. Diversification of culture media for production of entomopathogenic fungi. *Arquivos de Biologia O Tecnologia*. 39: 123 – 128

171953

Voronina, E. G., Gindina, G. M. and Novikova, I. T. 1987. Entomophthorous fungi in aphid control. *Zushchita Rastenii*. 6: 28 – 29

Walstad, J. D., Anderson, R. F. and Stambaugh, W. J. 1970. Effects of environmental conditions on two species of muscardine fungi (*Beauveria bassiana* and *Metarhizium anisopliae*). *J. Invertebr. Pathol.* 16: 221 – 226

Wickramatileke, W. S. N., Ahangama, D., Banda, D. M. G. A. 2000. Evaluation of the effectiveness of entomopathogenic fungus (*Metarhizium anisopliae* var. *major*) on cabbage semilooper (*Chrysodeixis erisoma* Doubt.). *Trop. agric. Res.* 12: 177 – 185

Zaki, F. N. 1998. Efficiency of the entomopathogenic fungus, *Beauveria bassiana* (Bals.), against *Aphis craccivora* Koch and *Bemisia tabaci*. *J. Appl. Ent.* 122: 397 – 399

Zentmeyer, G. A. 1955. A laboratory method for testing soil fungicides with *Phytophthora cinnammomi* a test organism. *Phytopathology* 45: 398 – 404

Zhang, A. W., Liv. W. Z., Nong, X. Q., Deng, C. I., Dus, W. L. and Lang, B. 1992. A trial production of wettable powder of *Beauveria bassiana*. *Chin. J. Biol. Control* 3: 118 – 120

Zimmermann, G. 1978. Isolation and new description of *Entomophthora blunckii* from the cabbage moth *Plutella maculipennis*. *Entomophaga* 23: 181 – 187

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*Originals not seen

EFFECT OF ENTOMOPATHOGENIC FUNGI ON SUCKING PESTS AND LEAF FEEDERS OF VEGETABLES UNDER *IN VITRO* CONDITIONS

JINCY SIMON

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Department of Agricultural Entomology
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM-695 522

ABSTRACT

Investigation on "Effect of entomopathogenic fungi on sucking pests and leaf feeders of vegetables under *in vitro* conditions" was carried out in the Insect Pathology Laboratory, Department of Agricultural Entomology, College of Agriculture, Vellayani.

Among the seven entomopathogenic fungi tested, *Beauveria bassiana* was found to infect *Sylepta derogata*, *Spodoptera litura* and *Pericallia ricini*, an aphid isolate of *Fusarium pallidoroseum* infected aphids *Aphis craccivora* and *Myzus persicae* while *Rhizopus oryzae* caused mortality of *Dysdercus cingulatus*.

The LC_{50} values for *B. bassiana* were calculated as 5.75 x 10^8, 6.99 x 10^8 and 2.72 x 10^5 spores ml^{-1} against *S. derogata*, *S. litura* and *P. ricini* respectively. *F. pallidoroseum* caused 50 per cent mortality of *A. craccivora* and *M. persicae* at 3.86 x 10^6 and 0.72 x 10^6 spores ml^{-1} respectively. *R. oryzae* caused 50 per cent mortality of *D. cingulatus* at 4.15 x 10^6 spores ml^{-1}.

Evaluation of different naturally available substrates for mass multiplication of *B. bassiana*, *F. pallidoroseum* and *R. oryzae* revealed that both rice bran and gingelly oil cake were suitable for the mass multiplication of these fungi. Among the liquid substrates, coconut water produced maximum mycelial growth of all the fungi.

At room temperature, *B. bassiana* retained the virulence upto five months after inoculation in rice bran and gingelly oil cake, but the virulence of the fungus was drastically reduced after two months of storage. *F. pallidoroseum* retained the virulence upto six months in rice bran and gingelly oil cake but *R. oryzae* lost the virulence after two months of storage. Coconut water and starch solution could not retain the viability and virulence of the fungi after two months of storage.

Studies conducted to evaluate the compatibility of *B. bassiana*, *F. pallidoroseum* and *R. oryzae* with different pesticides revealed that

two per cent neem oil emulsion could be incorporated with *B. bassiana* and *R. oryzae* without any inhibitory effect while sporultion of *F. pallidoroseum* was inhibited by neem oil. Mancozeb, dimethoate malathion, quinalphos and chlorpyriphos greatly inhibited the growth and sporulation of the three fungi tested.